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1.	Your reference	P0147-GB01
2.	Patent application number *(The Patent Office will fill in this part)*	0208331.9 11 APR 2002
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	OXFORD GLYCOSCIENCES (UK) LTD THE FORUM 86 MILTON PARK ABINGDON, OXON OX14 4RY UNITED KINGDOM
	Patents ADP number *(if you know it)*	71~~12386002~~ 7112386002
	If the applicant is a corporate body, give the country/state of its incorporation	ENGLAND AND WALES
4.	Title of the invention	Proteins
5.	Name of your agent *(if you have one)* "Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	BLAKEY, ALISON JANE OXFORD GLYCOSCIENCES (UK) LTD THE FORUM 86 MILTON PARK ABINGDON, OXON OX14 4RY UNITED KINGDOM
	Patents ADP number *(if you know it)*	~~7112386002~~ 7081219002

		Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*
6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number			

		Number of earlier application	Date of filing *(day / month / year)*
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8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
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Description	80
Claim *(s)*	5
Abstract	1
Drawing *(s)*	0

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Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature Alison Bloley Date 10-April-2002

12. Name and daytime telephone number of person to contact in the United Kingdom MARY GADSDEN 01235 208127

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PROTEINS

The present invention relates to the identification of membrane proteins not previously reported in human breast cancer cells which can form biological targets against which therapeutic antibodies or other pharmaceutical agents can be made, or have utility as diagnostic and prognostic markers for breast cancer and breast cancer metastases.

Breast cancer is the second leading cause of cancer death for women in the U.S; approximately 40,000 women in the US die from the disease each year. Breast cancer is the leading cause of cancer death for US women between the ages of 20 and 59, and the leading cause of cancer death for women worldwide. In the United Kingdom there are over 38,000 new cases each year accounting for more than one in four of all cancer cases in women. The lifetime risk of breast cancer in women in the United Kingdom is 1 in 9 (1 in 8 in the USA). Ninety-nine percent of breast cancers occur in women with the risk of development increasing with age. The annual cost of breast cancer treatment in the United States is approximately $10 billion (Fuqua, et. al. 2000, American Association for Cancer Research, www.aacr.org, USA). Breast cancer incidence has been rising over the past five decades, but recently it has plateaued. This may reflect a period of earlier detection of breast cancers by mammography. Mammography screening does not prevent or cure breast cancer; however, it may detect the disease before symptoms occur. Breast cancer tumors can exist for six to ten years before they grow large enough to be detected by mammography. In addition, mammography is less effective in younger women than in older women. Studies show that regular mammography screening of women between the ages of 50 and 69 reduces breast cancer mortality by approximately 30% in this age group. However, women with breast cancer will not benefit from mammography screening unless they have access to appropriate treatment. All women are at risk for breast cancer and approximately 90% of women who develop breast cancer do not have a family history of the disease.

Factors that increase a woman's risk of breast cancer include: older age, earlier age at menarche, later age at menopause, nulliparity, later age at first full-term pregnancy, daily alcohol consumption, long-term use of hormonal replacement therapy, postmenopausal obesity, infrequent physical activity, ionizing radiation, genetic factors and family history of breast or ovarian cancer. Although scientists have discovered some risk factors for breast cancer, most factors account for only small increases in a person's chances of developing breast cancer. There is no cure for breast cancer.

Causes of Breast Cancer

Breast cancer is a heterogeneous disease. Although female hormones play a significant role in driving the origin and evolution of many breast tumours, there are a number of other recognised and unknown factors involved. Perturbations in oncogenes identified include amplification of the HER-2 and the epidermal growth factor receptor genes, and overexpression of cyclin D1. Overexpression of these oncogenes has been associated with a significantly poorer prognosis. Similarly, genetic alterations or the loss of tumour suppressor genes, such as the p53 gene, have been well documented in breast cancer and are also associated with a poorer prognosis. Researchers have identified two genes, called BRCA1 and BRCA2, which are predictive of premenopausal familial breast cancer. Genetic risk assessment is now possible, which may enhance the identification of candidates for

chemoprevention trials (Fuqua, et. al. 2000, American Association for Cancer Research, www.aacr.org, USA).

Diagnosis

Early diagnosis of breast cancer is vital to secure the most favourable outcome for treatment. Screening typically takes the form of regular x-ray of the breast (mammography) and regular breast self-examination. Abnormalities detected during these screeening procedures and cases presenting as symptomatic would normally be confirmed by aspiration cytology, core needle biopsy with a stereotactic or ultrasound technique for nonpalpable lesions, or incisional or excisional biopsy. At the same time other information relevant to treatment options and prognosis, such as oestrogen receptor (ER) and progesterone receptor (PR) status would be determined (National Cancer Institute, USA, 2000, Breast Cancer PDQ, www.nci.nih.gov).

Disease Staging and Prognosis

Staging is the process of finding out how far the cancer has spread. A simplified set of stages is shown in Table A, labeled stage 0 through stage IV (0-4). In general, the lower the number, the less the cancer has spread. A higher number, such as stage IV (4), means a more serious cancer. (American Cancer Society, 2000, USA, www.cancer.org)

Table A.

Stage	5-year relative survival rate
0	100%
I	98%
IIA	88%
IIB	76%
IIIA	56%
IIIB	49%
IV	16%

(American Cancer Society, 2000, USA, www.cancer.org)

Although anatomic stage is an important prognostic factor, other characteristics may have predictive value. For example studies from the National Surgical Adjuvant Breast and Bowel Project (NSABP) and the International Breast Cancer Study Group (IBCSG) have shown that tumour nuclear grade and histologic grade, respectively, are important indicators of outcome following adjuvant therapy for breast cancer. There is substantial evidence that oestrogen receptor status and measures of proliferative capacity of the primary tumour may have important independent predictive value. In stage II disease, the PR status may have greater prognostic value than the ER status. Tumour vascularisation, c-erbB2, c-myc, p53 expression, and lymphatic vessel invasion may also be prognostic indicators in patients with breast cancer (National Cancer Institute, USA, 2000, Breast Cancer PDQ, www.nci.org and references therein).

Treatment

Surgery, radiation therapy, hormone therapy, and chemotherapy are the most common

treatments for breast cancer.

Surgery: Most women with breast cancer will have some type of surgery to remove as much of the cancer as possible. This may comprise a lumpectomy, axillary dissection or more radical mastectomy with breast reconstruction. Surgery may also be combined with other treatments like chemotherapy, hormone therapy, or radiation therapy.

Chemotherapy: Chemotherapy is the use of anticancer drugs to kill cancer cells and is most often given after surgery (adjuvant therapy) when it can reduce the chance of cancer recurrence. Chemotherapy can also be used as the main treatment for women whose cancer is widespread when it is found, or spreads widely after initial treatment. Neoadjuvant chemotherapy is given before surgery, often to shrink the tumour and make it easier to remove. Chemotherapy is often more effective when several drugs are combined, rather than a single drug alone. The most commonly used combinations are: cyclophosphamide, methotrexate, and fluorouracil (CMF) cyclophosphamide, doxorubicin (Adriamycin™), and fluorouracil (CAF) doxorubicin (Adriamycin) and cyclophosphamide (AC), with or without paclitaxel (Taxol™) doxorubicin (Adriamycin), followed by CMF.

Radiation therapy: Radiation therapy is commonly applied in breast cancer treatment to reduce the size of a tumour before surgery or to destroy cancer cells remaining in the breast, chest wall, or underarm area after surgery.

Hormone therapy and Chemoprevention: The hormone oestrogen can increase the growth of breast cancer cells in some women. A drug such as tamoxifen, which blocks the effect of oestrogen, is given to counter this growth. Another newer drug, raloxifene, also blocks the effect of oestrogen on breast tissue and breast cancer. There is increasing evidence that these anti-oestrogen treatments may also have a role in chemoprevention of breast cancer in high-risk individuals.

Immunotherapy: Trastuzumab (Herceptin™) is a new immunotherapeutic agent that attaches to a growth factor receptor known as c-erbB2/HER2/neu, which is present in small amounts on the surface of normal breast cells and at much higher levels in some breast cancers. This protein can cause the cancer to grow and spread faster. Herceptin can stop the c-erbB2/HER2/neu protein from promoting breast cancer cell growth. It may also help the immune system to better attack the cancer. Herceptin is currently started after standard hormonal or chemotherapy is no longer working (American Cancer Society, 2000, USA, www.cancer.org).

Therapeutic Challenges

The major challenges in breast cancer treatment are to improve early detection rates, to find new non-invasive markers that can be used to follow disease progression and identify relapse, and to find improved and less toxic therapies, especially for more advanced disease where 5 year survival is still very poor. There is a great need to identify targets which are more specific to the cancer cells, ideally ones which are expressed on the surface of the tumour cells so that they can be attacked by promising new approaches like immunotherapeutics and targeted toxins.

The present invention provides methods and compositions for screening, diagnosis, prognosis and therapy of breast cancer, for monitoring the effectiveness of breast cancer treatment, and for drug development for treatment of breast cancer.

We have used mass spectrometry to identify peptides generated by gel electrophoresis and tryptic digest of membrane protein extracts of human breast cancer-derived cell lines. Peptide sequences were compared to existing protein databases and to existing cDNA databases and

corresponding gene sequences identified. The identified proteins and proteins encoded by the identified nucleic acid sequence represent a set of proteins of potential diagnostic and/or therapeutic value.

Thus, one aspect of the invention provides methods for diagnosis of breast cancer that comprises analysing a sample of breast tissue by one-dimensional electrophoresis to detect at least one Breast Cancer-associated Membrane Protein as defined in Tables 1 or 2 (hereinafter referred to as BCMP) herein, or any combination thereof. These methods are also suitable for screening, prognosis, monitoring the results of therapy, drug development and discovery of new targets for drug treatment.

Another aspect of the invention provides methods of treating breast cancer, comprising administering to a patient a therapeutically effective amount of a compound that modulates (e.g. upregulates or downregulates) or complements the expression or the biological activity (or both) of a BCMP in patients having breast cancer, in order to (a) prevent the onset or development of breast cancer; (b) prevent the progression of breast cancer; or (c) ameliorate the symptoms of breast cancer.

Yet another aspect of the invention provides methods of screening for compounds that modulate (e.g. upregulate or downregulate) the expression or biological activity of a BCMP.

A further of the invention provides antibodies including, but not limited to polyclonal, monoclonal, bispecific, humanized or chimeric antibodies, single chain antibodies, Fab fragments and F(ab') fragments, fragments produced by a Fab expression library, anti-diotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that specifically binds an antigen. The immunoglobulin molecules of the invention can be of any class (*e.g*, IgG, IgE, IgM, IgD and IgA) or subclass of immunoglobulin molecule capable of immunospecific binding to a BCMP.

The present invention also provides a method for screening for and/or diagnosis of breast cancer in a human subject, which method comprises the step of identifying the presence or absence of one or more BCMPs, in a biological sample obtained from said human subject.

Yet another aspect of the present invention provides a method for monitoring and/or assessing breast cancer treatment in a human subject, which comprises the step of identifying the presence or absence of one or more BCMPs, in a biological sample obtained from said human subject.

In a further aspect, the present invention provides a method for identifying the presence or absence of metastatic breast cancer cells in a biological sample obtained from a human subject, which comprises the step of identifying the presence or absence of one or more BCMPs.

In another aspect, the present invention provides a method for monitoring and/or assessing breast cancer treatment in a human subject, which comprises the step of determining whether one or more of the BCMPs is increased/decreased in a biological sample obtained from a patient.

The biological sample used can be from any source such as a serum sample or a tissue sample, e.g. breast tissue. For instance, when looking for evidence of metastatic breast cancer, one would look at major sites of breast metastasis, e.g. lymph nodes, liver, lung and/or bone.

Preferably, the methods of the present invention are not based on looking for the presence or absence of all of the BCMPs, but rather on "clusters" or groups thereof.

Other aspects of the present invention are set out below and in the claims herein.

The invention described in detail below provides methods and compositions for clinical screening, diagnosis and prognosis of breast cancer in a mammalian subject for identifying patients most likely to respond to a particular therapeutic treatment, for monitoring the results of breast cancer therapy, for drug screening and drug development. The invention also encompasses the administration of therapeutic compositions to a mammalian subject to treat or prevent breast cancer. The mammalian subject may be a non-human mammal, but is preferably human. For clarity of disclosure, and not by way of limitation, the invention will be described with respect to the analysis of breast tissue samples. However, as one skilled in the art will appreciate, the assays and techniques described below can be applied to other types of patient samples, including a body fluid (*e.g.* blood, serum, plasma or saliva), a tissue sample from a patient at risk of having breast cancer (*e.g.* a biopsy such as a breast tissue biopsy) or homogenate thereof. The methods and compositions of the present invention are specially suited for screening, diagnosis and prognosis of a living subject, but may also be used for postmortem diagnosis in a subject, for example, to identify if family members are at risk of developing the same disease.

As used herein, breast tissue refers to the breast itself, as well as the tissue adjacent to and/or within the strata underlying the breast.

Breast Cancer-associated Membrane Proteins (BCMPs)

In one aspect of the invention, one-dimensional electrophoresis is used to analyse breast tissue from a subject, preferably a living subject, in order to measure the expression of one or more Breast Cancer-associated Membrane Proteins (BCMPs) for screening, prognosis or diagnosis of breast cancer, to monitor the effectiveness of breast cancer therapy, or for drug development. As used herein, "one-dimensional electrophoresis" (1D-electrophoresis) means a technique comprising denaturing electrophoresis; this generates a one-dimensional gel (1D-gel) containing a plurality of separated proteins. Preferably, the step of denaturing electrophoresis uses polyacrylamide electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE). A one-dimensional array (analogous to the two-dimensional array generated using the technology of US Patent No. 6,278,794) is generated by separating biomolecules on a one-dimensional gel according to their electrophoretic mobility. A computer-generated digital profile of the array is generated, representing the identity, apparent molecular weight of a plurality of biomolecules detected in the one-dimensional array, thereby permitting computer-mediated comparison of profiles from multiple biological samples, as well as computer aided excision of separated proteins of interest.

As used herein, the term "Breast Cancer-associated Membrane Protein" (BCMP) refers to a feature (*e.g.* a band in a 1D-gel) detectable by 1D-electrophoresis of a breast tissue sample, and present in breast tissue from a subject having breast cancer.

The BCMPs disclosed herein have been identified in membrane protein extracts of human breast cancer-derived cell lines separated generally using 1D-gel electrophoresis and tryptic digest of membrane protein extracts of human breast cancer-derived cell lines). Peptide sequences were compared to the SWISS-PROT and trEMBL databases (held by the Swiss Institute of Bioinformatics (SIB) and the European Bioinformatics Institue (EBI) which are available at http://www.expasy.com/) and the GenBank database (held by the National Institute of Health (NIH) which is available at http://www.ncbi.nlm.nih.gov/GenBank/) and corresponding genes identified. Published reports and databases, including databases of proteins expressed in normal human breast luminal epithelial cells

(Page *et al.*, Proc Natl Acad Sci U S A. 1999 96(22): 12589-94; GB patent application no. 9919258.5) were searched to establish whether the products of any of the identified genes had been previously demonstrated to be expressed in the membrane of human breast cells or human breast cancer cells. Two groups of BCMPs have been identified: (1) protein sequences matching conceptual translations of cDNAs for which no protein or biological function has been described, for which the present invention defines the existence of the protein product and its localisation in the membranes of human breast cancer cells; (2) known proteins which have not previously been described in breast cell membranes, which the present invention shows may be additionally involved in human breast cancer. All of the BCMPs find utility as markers for breast cells, especially breast cancer cells. The amino acid sequences of identified tryptic digest peptides of those proteins falling into the first category are shown in Table 1 below, and those in the second category are shown in Table 2 below. Each protein in Tables 1 and 2 is identified by a Swiss Prot or a Genbank Accession Number and each sequence is incorporated herein by reference, or the sequences correspond to novel polypeptides. The apparent molecular weight and the amino acid sequences of tryptic digest peptides of these BCMPs identified by tandem mass spectrometry and database searching as described in the Examples, *infra*, are also listed in these Tables.

Table 1. Protein sequences matching conceptual translations of cDNAs or genes for which no protein or biological function has been described

Table 1 BCMP No.	MW (kDa)	Amino Acid Sequences of Tryptic Digest Peptides	Accession Nos. of Identified Sequences
BCMP-1001	45.7	AQYEDLANR, LEGLTDELNFLR, LSELEAALQR, QLYEEELR	Q9H552
BCMP-1002	39.8	LTGPAAAEPR	CAC35001
BCMP-1003	153.4	LLATEQEDAAVAK	Q9H475
BCMP-1004	42.6	GHDWGAPPFR	Q9UGT4
BCMP-1005	41.5	QLEQQEELLR	Q9NUY7
BCMP-1006	25.7	TEEGLGFNLMGGK	Q9NUP9
BCMP-1007	28.8-29.1	LLDETQEAVEYQR, MHQVMSLEEVER	Q9HAE8
BCMP-1008	50.6-196.9	ALQVADFSGNPLTR, LGLSDNELQR, SLEELLLDANQLR	Q9HAC0
BCMP-1009	105.9	LNLVGGMFDTVQR	Q9H9M5
BCMP-1010	30.5	NLLLTNEQLESAR, VGLPVTDENGNR	Q9H9B4
BCMP-1011	45.0	LSLPGQMAGTPLTPLK	Q9H8Y8
BCMP-1012	45.0-47.9	SMLQATAEANNLAAAASAK, DLDVVVVSVAGAFR, EHQHEELQNVR	Q9H8Q5

Table 1 BCMP No.	MW (kDa)	Amino Acid Sequences of Tryptic Digest Peptides	Accession Nos. of Identified Sequences
BCMP-1013	28.9-29.3	APTASADAELSAQLSR, LSELDVALEGVK, LGAPQTHLGLK	Q9H8I2
BCMP-1014	50.6	GPDALTLLEYTETR	Q9H8G0
BCMP-1015	110.5	EELNKPPLAK	Q9H7V0
BCMP-1016	34.4	ALGTAYAVLSNPEK	Q9NXW2
BCMP-1017	115.6	GDLEVLQAQVER	Q9NXV1
BCMP-1018	38.3	AMSNDGHLECAR	Q9NXJ6
BCMP-1019	31.4	ANLVMLVPK	CAA75875
BCMP-1020	62.3	HDDDELGFR	O95513
BCMP-1021	34.4-291.0	GEVTEMFSYEESNPK, NASDMPETLTSR	Q9H111
BCMP-1022	51.3	FELDTSER, FSSGYYDFLVEVEGDNR, QELQHLFR, SLVEELEDLVAR, YLANTVELR	Q9Y3K6
BCMP-1023	40.6-41.5	LENGELETLAR, YSLVLELSDSGAFR, LLLSSETPLEGK	Q9NUB2
BCMP-1024	40.6-41.1	EEADYSAFGTDTLLK	Q9Y510
BCMP-1025	52.9	LVSDEAVTNGLR	novel
BCMP-1026	354.7	AEGPDVAVDLPK, GPEVDVNLPK	novel
BCMP-1027	67.6	EGNELQFLQLVK	AAG50293
BCMP-1028	87.9	GEWCMVGGCGQPQVGAGR	Q9H7J3
BCMP-1029	105.3-109.9	NVFYELEDVR, SPGVLFLQFGEETR	novel
BCMP-1030	29.1	YQYALDEYYR	novel
BCMP-1031	26.7-27.0	ELLEQMDLEVR, AHLLDNTER	novel
BCMP-1032	50.6	WPFSLSEQQLDAR	novel
BCMP-1033	28.1	YASEDAELLLVGNK	novel
BCMP-1034	85.2-94.6	RTPDGFDSVPLK, TPDGFDSVPLK	novel
BCMP-1035	49.2	YCQPEEEVAR	novel
BCMP-1036	34.7-35.3	APPNATLEHFYLTSGK, QVEVELLSR	novel
BCMP-1037	29.3	GSEQDVVLDTGLGLR	novel
BCMP-1038	62.7	AAVMVYDDANK	novel
BCMP-1039	29.7	QLYLDVESVR	novel
BCMP-1040	65.3	AAEGWSAPLLTLAR	novel
BCMP-1041	46.1	EPEQPPALWR	novel
BCMP-1042	26.8	LEVLVNFMR	novel

Table 1 BCMP No.	MW (kDa)	Amino Acid Sequences of Tryptic Digest Peptides	Accession Nos. of Identified Sequences
BCMP-1043	94.6	GWEFHSVELNR	novel
BCMP-1044	291.0	ELELAELDQWPQER	novel
BCMP-1045	53.7-55.4	LMVPVTVVFTR, DGAPVATNAFHSPR	novel
BCMP-1046	66.7	LEGAELEEFLR	novel
BCMP-1047	31.6-32.8	LQQLPADFGR, LVNLQHLDLLNNK	novel
BCMP-1048	90.8	SLPLYSPEK	novel
BCMP-1049	7.4-94.0	LGNLEFKPESR, VNGVDDAANFR	novel
BCMP-1050	179.3	GQGTEFNLTFR	novel
BCMP-1051	49.2-49.9	LAQAWFNTHR, RQDLLNVLAR, RLEEAALR	Q9NRG3
BCMP-1052	31.2	LNDTQLDSGSPLR	O94779
BCMP-1053	83.3-88.5	AVLGDDELTR, DYHFVTSR, RDYEVDGR	AAA50599
BCMP-1054	37.6-50.6	EPELFQTVAEGLR, HLLEQDFPGMR	O14611
BCMP-1055	31.6-31.9	YGEDSEQFR	Q9H2P8
BCMP-1056	196.0	ELPTAFDYVEFTR, GDSLDSVEALLK, LLQSHPESAEDLQEK	Q9UG16
BCMP-1057	26.0	MGPGAASGGERPNLK	Q9H0U4
BCMP-1058	25.2	VNLNQLPLGR	O60648
BCMP-1059	249.0	ESTLHLVLR	Q9UFQ0
BCMP-1060	31.4-32.8	NPGPSGPQLR, QLEADLLDVNQLFK, ELGSLPLPLSTSEQR	O95564
BCMP-1061	30.7-30.9	FLMGTNSPDSR, GASWLDTADGSANHR, SVLWWLPVEK	Q9Y405
BCMP-1062	32.1	YGEDSEQFR	Q9UG89
BCMP-1063	67.6-70.8	FQELLFEDFAR, VEGPAFTDALR	Q9NT35
BCMP-1064	62.3-63.5	SEDPDQQYLLLNTAR	Q9H096
BCMP-1065	49.2	AVSDWLLASVEGR	CAB66502
BCMP-1066	28.3	DQALSLSAMEELPR	O60811
BCMP-1067	35.0-35.3	LSELEDAAFLAR, DLNLMAPGLTLQAVR	O75477
BCMP-1068	219.2	FSSPDELDLPR, LQTPELGEVFQNK, QFDVNLQVPDR	Q92580
BCMP-1069	25.5-25.6	RPSAPVDFSK	Q14696

Table 1 BCMP No.	MW (kDa)	Amino Acid Sequences of Tryptic Digest Peptides	Accession Nos. of Identified Sequences
BCMP-1070	85.8-91.4	NFPQTALVSFATTGEK, RPELPTEQSR, VLPTQPNPVDASR	Q14700
BCMP-1071	69.2	GSLGPTVLEVFNTLLK	Q14156
BCMP-1072	165.2-179.3	LPLLPPESPGPLR, VLSLNGVDVTEAR, ALWLAENQAQPMLR	Q14160
BCMP-1073	44.4	VLTLVSQLDVNNEFEK	Q92621
BCMP-1074	62.7-142.8	ALTELYLTR, HALLEEENR, AEEASHWLWSR, LQPETGPLGGGLR, LQLEQQVATGPALDNK	O15031
BCMP-1075	291.0	DATFHYGEQAAK	O75054
BCMP-1076	36.9-38.7	EDTESLELFQNEVAR, EEFPNENQVVFAR	O60319
BCMP-1077	179.3	ELLVDLLDASAAK, YFFDFLEEQAEK	Q9Y4D7
BCMP-1078	51.3-164.4	RADTVGLACEALNR, VLHALSENELCVR, TLTMETSDDLLFSK	O75165
BCMP-1079	82.7-85.8	HQVEYLGLLENVR, LMYNSSNPVLK, NYDTTLHGK, VLVQQPGER, RPLTAATLFK, SYLHEVAR, SLPASDLPQVR	O94832
BCMP-1080	7.4-94.6	FEWELPLDEAQR, ALTLGALTLPLAR, ELPAWVSFPDVEK, LLAETVAPAVR, LTLWYYSEER, VQLDLAETDLSQGVAR	O94848
BCMP-1081	69.2	QNGYLEFDALSR	O94874
BCMP-1082	25.1	SHYLADSDPLLSK	O94929
BCMP-1083	52.9	TNVLQSLLAR	O94935
BCMP-1084	115.6	YLDLEPDTPLSPEELK	Q9Y2H5
BCMP-1085	35.6	DPNNQLHVLK, LNHLSFAELLKPFSR	Q9Y2L5
BCMP-1086	34.4-35.6	SEANDQGPPGEDGVTR	Q9ULQ8
BCMP-1087	29.5-30.5	YSYQYTVANK, EYVAYSHTGR	Q9ULN9
BCMP-1088	57.3	TAATLATHELR	Q9ULN5

Table 1 BCMP No.	MW (kDa)	Amino Acid Sequences of Tryptic Digest Peptides	Accession Nos. of Identified Sequences
BCMP-1089	94.6	AGVSSQPVSLADR, LCSGGLSLPEQR	Q9P2M2
BCMP-1090	165.2	LLATEQEDAAVAK	Q9P2E9
BCMP-1091	109.9	FSSVSSPQPR	Q9P206
BCMP-1092	37.6	LLAELESGGGSVPPMK	BAB21775
BCMP-1093	165.2	TLLTYAEEDLELR	BAB21862
BCMP-1094	28.5	AVTEQGAELSNEER, AVTEQGHELSNEER, NLLSVAYK, SVTEQGAELSNEER, YLLPNATQPESK	AAF19232
BCMP-1095	28.7	MSVSSLSSLTSLK	Q92698
BCMP-1096	50.6	LESVHFSDQFSGPK, RQDLLNVLAR	Q9H3E4
BCMP-1097	32.8	QVFEGLTTGLLK	AAG47945
BCMP-1098	45.7	LGEAEATVLEAMGK	AAH03683
BCMP-1099	40.6-45.7	EYVESQLQR, LFKPGQEAVK, LQPTWNDLGDK	AAH01199
BCMP-1100	52.9	SSSAGGQGSYVPLLR	AAH04381
BCMP-1101	45.2-47.3	DGEEAGAYDGPR, FVMQEEFSR, LAPEYEAAATR, QAGPASVPLR, DLLLAYYDVDYEK, ELSDFLSYLQR, FLQDYFDGNLK, LNFAVASR, MDATANDVPSPYEVR	XP_016522
BCMP-1102	45.7	DGEEAGAYDGPR, DLLLAYYDVDYEK, ELSDFLSYLQR, FLQDYFDGNLK, FVMQEEFSR, MDATANDVPSPYEVR, VVVAENFDELVNNENK	XP_007536
BCMP-1103	24.9-25.1	HVVFLAQR	XP_017030
BCMP-1104	24.2-24.4	SMLDQLGVPLYAVVK	AAH05871
BCMP-1105	27.3	GLHEDLQGVPER	AAH01598
BCMP-1106	31.2	GASWLDTADGSANHR, MGNTPDSASDNLGFR	AAH06159
BCMP-1107	25.5	HNWGQGFR, NFLSTPQFLYR	AAH02457
BCMP-1108	24.3-25.0	EAPVDVLTQLGR, RDFAPPGQQK	AAH01309
BCMP-1109	29.1	ALVFVVDSAAFQR, LADGGGAGGTFQPYLDTLR, VADGGGAGGTFQPYLDTLR	BAB55176

Table 1 BCMP No.	MW (kDa)	Amino Acid Sequences of Tryptic Digest Peptides	Accession Nos. of Identified Sequences
BCMP-1110	28.7	MSVSSLSSITSLK	XP_001604
BCMP-1111	24.1-24.4	SMLDQLGVPLYAVVK	AAK55527
BCMP-1112	28.3	DQALSISAMEELPR	Q9UQP2
BCMP-1113	39.8	CVCNPAGCLR, HCVATVCGQTCTNPVK	Q9H318
BCMP-1114	24.3-24.4	NVLGLQMGTNR	Q9H4P0
BCMP-1115	32.8	QDVDDEYGVSQALAR, TIQTIALITYLMEHK, EEDEVPDDETVNQMIAR	P51531
BCMP-1116	32.1	MGPSGGEGMEPER, QQSELQSQVR, YHEEFEK	Q9NPR7
BCMP-1117	30.5	NLLLTNEQLESAR	Q9HA53
BCMP-1118	91.3	TTAFSVWTK	AK026832
BCMP-1119	29.0	SNPEDQILYQTER	Q92508
BCMP-1120	32.3	QVFEGLTTGLLK	Q9H3D8
BCMP-1121	24.1	SMLDQLGVPLYAVVK	Q96JR0
BCMP-1122	66.6	LEGAEIEEFLR	AAH10668
BCMP-1123	35.3	APPNATLEHFYLTSGK	Q96G23
BCMP-1124	32.5	LQQLPADFGR, LVNLQHLDLLNNK	AK025328
BCMP-1125	46.9	NPALYASNVR	AK022499
BCMP-1126	24.5	EAPVDVLTQIGR	Q9BVC6
BCMP-1127	25.4	NFLSTPQFLYR, HNWGQGFR	Q9BUN8
BCMP-1128	30.9	FLMGTNSPDSR, SVLWWLPVEK, GASWIDTADGSANHR	Q9BRN3
BCMP-1129	24.1	AGGGGGLGAGSPALSGGQGR, YASEDAELLLVGNK	novel
BCMP-1130	26.8	LEVLNVFMR	novel
BCMP-1131	109.8	SPGVLFLQFGEETR	Q9QWI6

Table 2. Known proteins which have not previously been described in breast cell membranes

Table 2 BCMP #	MW (kDa)	Amino Acid Sequences of Tryptic Digest Peptides	Accession Nos. of Identified Sequences
BCMP-1132	26.4	NYGQLDLFPAR	Q14876

Table 2 BCMP #	MW (kDa)	Amino Acid Sequences of Tryptic Digest Peptides	Accession Nos. of Identified Sequences
BCMP-1133	82.7-85.8	DTPDEPWAFPAR, SSHYDELLAAEAR, EADGSETPEPFAAEAK, LGTLSPAFSTR, SEAVVEYVFSGSR	Q13122
BCMP-1134	85.8	LAVQQVEEAQQLR	O00461
BCMP-1135	28.0-28.7	AVTEQGHELSNEER, YLLPNATQPESK	P31946
BCMP-1136	28.1-28.3	NSVVEASEAAYK	Q04917
BCMP-1137	28.3-28.4	NVTELNEPLSNEER	Q9UN99
BCMP-1138	28.1-28.3	AVTEQGAELSNEER	P27348
BCMP-1139	27.8-28.4	SVTEQGAELSNEER	P29312
BCMP-1140	101.2-115.6	LYQPEYQEVSTEEQR, EAGMQPQLQLR, EVEEEPGLHSLK, QADNPHVALYQAR, AEAGPEGVAPAPEGEK	Q9Y4L1
BCMP-1141	28.0-28.2	LTQEQVSDSQVLLR, SLYSLLLGQDNAADQSR	Q9P0I2
BCMP-1142	29.9-30.1	GPFTDVVTTNLK	Q9P0L0
BCMP-1143	28.2-28.6	ELAEDGYSGVEVR	P23396
BCMP-1144	30.3	APAMFNLR	P49241
BCMP-1145	29.1	GLPHLVTHDAR	P12750
BCMP-1146	29.3-29.7	DLPGLTDTTVPR	P10660
BCMP-1147	39.8-42.0	LYGPSSVSFADDFVR, DEEVHAGLGELLR	P29043
BCMP-1148	52.1	AGAFEHLPSLR	Q13641
BCMP-1149	27.1-27.5	LATQLTGPVMPVR. STESLQTNVQR	P26373
BCMP-1150	26.6-27.5	LVALVDVLDQNR	P50914
BCMP-1151	26.1	RNPDTQWLTK, SLQSVAEER	P39030
BCMP-1152	24.4-25.5	LLTFDQLALDSPK, TAVVVGTLTDDVR	Q07020
BCMP-1153	40.2-41.1	HGSLGFLPR	P39023
BCMP-1154	42.1	LDELYGTWR	P36578
BCMP-1155	31.2	EFNAEVHR, GAVDGGLSLPHSTK, RFPGYDSESK	P46777
BCMP-1156	31.9-34.4	ALPQLQGYLR, HQEGELFDTEK, QLASGLLLVTGPLVLNR, YYPTEDVPR	Q02878

Table 2 BCMP #	MW (kDa)	Amino Acid Sequences of Tryptic Digest Peptides	Accession Nos. of Identified Sequences
BCMP-1157	28.2-28.9	AGNYFVPAEPK, LALTDNALLAR, QLFNGTFVK, TTHFVEGGDAGNR	P18124
BCMP-1158	29.5	ASGNYATVLSHNPETK	P25120
BCMP-1159	24.3	FLDGLYVSEK	P32969
BCMP-1160	53.7-64.0	VTHAVVTVPAYFNDAQR, DAGTLAGLNVMR, DNHLLGTFDLTGLPPAPR, FEELNMDLFR, KSDLDELVLVGGSTR, LTPSYVAFTPEGER, SDLDELVLVGGSTR, TWNDPSVQQDLK, VELLANDQGNR, VMEHFLK	P11021
BCMP-1161	52.1-52.9	ALDTLYQTTDFSGLR	O14672
BCMP-1162	83.3	DLPNENEAQFQLR, LQSSNLFTVAK	Q10567
BCMP-1163	76.2-78.7	YLETADYALR, FLNLFPETK	O95782
BCMP-1164	72.5	QLSNPQQEVQNLFK	O94973
BCMP-1165	76.7	LQNNNVYTLAK	P21851
BCMP-1166	41.1	EEVEGRPPLGVK	Q9Y6Q5
BCMP-1167	29.1	DFLAGGVAAALSK	P05141
BCMP-1168	28.8	AAYFGLYDTAK, DFLAGGVAAALSK, LLLQVQHASK	AAA36749
BCMP-1169	153.4-472.2	GEGPDVDVNLPK, GEGPEVDVTLPK, LPTGQLSGPELK, ADVDLSGPK, LSMPDLDLNLK, LSMPEVDLNLK, VHAPGLNLSGVGGK, VDLETPNLEGTLTGPR	Q13727
BCMP-1170	59.3	MYDDALEALEK	O60476
BCMP-1171	42.4-43.6	AAVPSGASTGLYEALELR, LGAEVYHNLK, GNPTVEVDLFTSK, VVLGMDVAASEFFR	P06733

Table 2 BCMP #	MW (kDa)	Amino Acid Sequences of Tryptic Digest Peptides	Accession Nos. of Identified Sequences
BCMP-1172	29.6-121.3	NAGNEQDLGLQYK, LLLLADMADVYK, ALMAQLPQEQK, LAEQVASFQEEK, QALQDLLSEYMGNAGR, QDLLAYLQR, QLLVDPLSFSEER, TSVQTEDDQLLAGQSAR, LAQENMDLFK	P35221
BCMP-1173	51.3-52.1	LPEGPLDQGPALGR	Q9P2U6
BCMP-1174	45.0	NEALAPPLLDAEPAR, SLVYQLNFDQTLR	Q10469
BCMP-1175	49.2	VFSNGADLSGVTEEAPLK	P01009
BCMP-1176	74.7-83.3	AGTQLENLDEDFR, ASLHEAWTDGK, GLSQEQMQEFR, HEAFESDLAAHQDR, HTNYTMEHLR, LASDLLEWLR, LSNRPAFMPSEGK, MLDAEDLVNTARPDEK, MLDAEDLVNTARPDEK, TLNEVENQLLTR, DHGGALGPEEFK, DYETATLSDLK	P12814
BCMP-1177	26.2-78.7	VGWEQLLTTLAR, AGTQLENLDEDFR, ASLHEAWTDGK, DDPLTNLNTAFDVAEK, DHGGALGPEEFK, LASDLLEWLR,	O43707
BCMP-1178	105.9-115.6	DSVLNLSESVEDGPK, LLAENNELLSNLR, YLVVYNPLEQDR	Q16706
BCMP-1179	71.2	QQYQNGLLASR	Q9NRN1
BCMP-1180	28.9-30.3	ELQTTTGNQQVLVR, VVVVTGANTGLGK, LANLLFTQELAR	Q9NRW0
BCMP-1181	24.8	FEEDVEEETER	P04920
BCMP-1182	165.2	NATTDALTSVLTK	Q12955
BCMP-1183	29.7-32.8	TPAQYDASELK, SYSPYDMLESLR, TNQELQELNR	XP_015855
BCMP-1184	29.8	LSQTYQQQYGR	P09525
BCMP-1185	115.0	TLLQCALNRPAFFAER	P20073

Table 2 BCMP #	MW (kDa)	Amino Acid Sequences of Tryptic Digest Peptides	Accession Nos. of Identified Sequences
BCMP-1186	29.7-32.8	AEDGSVLDYELLDQDAR, TNQELQELNR, WLSLMTER, DLYDAGVK, SYSPYDMLESLR, TPAQYDASELK	P07355
BCMP-1187	27.3	LPFPLLDDR	P30041
BCMP-1188	44.1-45.2	LLGADTSVDLEETGR, EAYPGDVFYLHSR, TGALVDVPVGEELLGR	P25705
BCMP-1189	43.6-44.1	FTQAGSEVSALLGR, LPVGPETLGR, LVLEVAQHLGESTVR, VALTGLTVAEYFR, VALVYGQMNEPPGAR	P06576
BCMP-1190	25.1	TDPSLLGGMLVR, VAASVLNPYVK	P48047
BCMP-1191	143.6	LGDELLELNGETTK	O75085
BCMP-1192	31.8	LVQAEGEAEAAK	Q99623
BCMP-1193	29.1-29.3	VLAAEGEMNASR, VQNATLAVANLTNADSATR, EASMVLTESPAALQLR	P27105
BCMP-1194	26.4-31.8	LQAAVDGPMDK, LVQAEGEAEAAK	P51572
BCMP-1195	52.9-76.1	NEGVATYAAAVLFR, MSEDKPQDYK, SPQMVSALVR, LLNDEDQVVVNK, TSMGGTQQQFVEGVR, AAVMVHQLSK	P35222
BCMP-1196	153.4-291.0	LGNLQTDLSDGLR, YADEELPR, AWGPGLHGGLVGR, SPFTVGVAAPLDLSK, TGEEVGFVVDAK, TFEMSDFLVDTR	O75369
BCMP-1197	248.1	LESEGSPETLTNLR	Q9NQA7
BCMP-1198	38.7	ESEPQAAAEPAEAK	P80723
BCMP-1199	41.2-45.7	DYLLLVMEGTDDGR, LENGELETLAR	Q9HDC9-
BCMP-1200	196.0-248.1	GPLVPLNVADQK, SELFNENFGPDFR	P55011
BCMP-1201	37.9-38.7	DLQEDSGMEPR	O75844
BCMP-1202	30.3	EPLLPPEDDTR	P12830

Table 2 BCMP #	MW (kDa)	Amino Acid Sequences of Tryptic Digest Peptides	Accession Nos. of Identified Sequences
BCMP-1203	28.0	THYSNLEANESEEVR	P04632
BCMP-1204	179.3	TSPNEGLSGNPADLER	P20020
BCMP-1205	30.9-69.2	APVPTGEVYFADSFDR, LPNPDFFEDLEPFR, LVDDWANDGWGLK, KDDTDDELAK, TPYTLMFGPDK, TPELNLDQFHDK, KLPNPDFFEDLEPFR, AEEDELLNR,	P27824
BCMP-1206	42.0-44.2	EQFLDGDGWTSR, GLQTSQDAR, VHVLFNYK	P27797
BCMP-1207	28.3-29.4	STLNELYFGK, LEVEANNAFDQYR, RLPPQQLEK, SGSGTMNLGGSLTR	P47756
BCMP-1208	142.8-165.2	DLSAENGLESLMLR, ELPNFWEQNR, LVGNMHGDETVSR, KNPAVTQLVDR, LVLVPSLNPDGR, QHHDEYEDELR, YYHEEELESALR	O75976
BCMP-1209	33.6-44.4	AAGDVDLGDAAYYFER	A48149
BCMP-1210	49.9	QGVGAGAGAPFGSSLAPTAR	XP_012772
BCMP-1211	31.2-31.4	TVLMLADQMLSR	P48729
BCMP-1212	50.6	AFYVNVLNEEQR	P04040
BCMP-1213	33.6-38.7	HTLADNFNPVSEER	P20645
BCMP-1214	179.3-249.0	DGAGNSFDLSSLSR, GHQAFDVGQPR	P11717
BCMP-1215	60.4	ELAQLVEDVQR	P39880
BCMP-1216	59.3	ESGVFEGLPTYR	XP_006738
BCMP-1217	59.3	QAELEELYESSLR	O00718
BCMP-1218	42.0	LSGKPQNAPEGYQNR, NVFLNQTR	Q9UQI9
BCMP-1219	109.9	LLEEQLQHELSNK	Q13999
BCMP-1220	115.0	LHLVDLAGSER	O95239
BCMP-1221	54.5-57.3	VAEQVGLDR, LTAAQHSVTGSAVSK	Q13492
BCMP-1222	42.9	LNYSDHDVLK	P20172
BCMP-1223	35.6-143.6	AFMTADLPNELLELLEK, LYLDSNNNPER, EHLELFWSR, NNLAGAEELFAR, RPLSADSALMNPASK, VGYTPDWLFLLR, LLYNNVSNFGR	Q00610

Table 2 BCMP #	MW (kDa)	Amino Acid Sequences of Tryptic Digest Peptides	Accession Nos. of Identified Sequences
BCMP-1224	120.7-142.9	LVLDNSVFSEHR, NNLAGAEELFAR, VGYTPDWLFLLR	P53675
BCMP-1225	68.1-69.6	GPAPQDQAGPGGAPR	O96005
BCMP-1226	115.6	LSFLYLLTGNLEK, SLLLSVPLLVVDNK	P53621
BCMP-1227	74.8	QYPLVTPNEER	P35606
BCMP-1228	127.8	DADSLTLFDVQQK, QELLLSNSEDK	AAB70879
BCMP-1229	40.2-41.5	DEEVHAGLGELLR, DTQSGSLLFLGR, LYGPSSVSFADDFVR, AVLSAEQLR	P50454
BCMP-1230	54.5	GTWTQPFDLASTR	P08185
BCMP-1231	41.2-45.7	TQYNQVPSEDFER	P78310
BCMP-1232	46.1	YLQDNPASGEK	Q16850
BCMP-1233	105.9-121.3	VEELEMTEDLR, MTALLFSDYR, QLTQNTDYR, VAELSSDDFHLDR	Q13221
BCMP-1234	24.8	GVNTGAVGSYLYDR	P52943
BCMP-1235	39.8	CVCNPAGCLR, HCVATVCGQTCTNPVK	Q9NZV1
BCMP-1236	28.7-29.1	MQGWSEVFQSR	O75881
BCMP-1237	25.7	HMATTQDEVHTK, LEWVCQLPK	O60449
BCMP-1238	29.4	YQETFNVLER	Q13011
BCMP-1239	49.9-115.6	VVPSFLPVDQGGSLVGR	Q14126
BCMP-1240	24.8-196.9	SVQNDSQALAEVLNQLK, LNDSLLQATEQR, LTVDSALAR, QLQNLLQATSR, ALLQALLQTEDMLK	P15924
BCMP-1241	24.6-26.6	ESSETPDQFMTADETR, SQEMVHLVNK	Q9H5A7
BCMP-1242	34.4	LTDAFLLLR, QLVQSLSDLNELFR	Q9UIX5
BCMP-1243	28.2-28.8	QLNYTLGEVPLSFVDR, YSVLLPTYNER, EGNFDLVSGTR,	O60762
BCMP-1244	39.8-40.6	YSQTGNYELAVALSR, NTLLLAGLQAR, TLVLLDNLNVR, WVPFDGDDLQLEFVR,	P39656
BCMP-1245	37.6-165.2	QELQHLFR, NPLLWNVADVVLK, SLVEELEDLVAR	P04844

Table 2 BCMP #	MW (kDa)	Amino Acid Sequences of Tryptic Digest Peptides	Accession Nos. of Identified Sequences
BCMP-1246	53.7-472.2	NLELDSPYELSR, TLLPAAAQDVYYR, VTAEVVLAHLGGGSTSR, ALTSELALLQSR, GEDEEENNLEVR, HFDETVNR, SEDLLDYGPFR,	P04843
BCMP-1247	70.8-76.7	LELLAAYEEVLR, YVLLNWVGEDVPDAR, EQSLFGDHR	Q16643
BCMP-1248	51.3-52.9	EHQLSPGDFPSLR, ELVNNLGELYQK, EPELFQTVAEGLR, LLPLEEHYR, LFEAEEQDLFK, LNAFGNAFLNR	Q9UNR3
BCMP-1249	31.4-42.1	LGGLGTVPVGR, LPLQDVYK, YYVTLLDAPGHR, SGDAALVDMVPGK, YYLTLLDAPGHR	P04720
BCMP-1250	71.2-76.7	YEWDVAEAR, EDLYLKPLQR, EGLPALDNFLDK	P13639
BCMP-1251	30.5	APGAGLGQDR, KLFEYETQR, LFEYETQR, TYGEPESAGPSR, YNLPHGPVVGSTR	P50402
BCMP-1252	27.9-28.2	FDTQYPYGEK, LLDQGEDFPASEMTR, WAEQYLK, DGDFENPVPYTGAVK, SLNLLTAFQK	P30040
BCMP-1253	43.9	AAFENWEVEVTFR	Q9UQG1
BCMP-1254	72.5-76.7	EEEALQLDGLNASQLR, KEAESSPFVER	P14625
BCMP-1255	291.0	QEVQDLQASLK	Q15075
BCMP-1256	127.1-354.7	LANLQTDLSDGLR, AFGPGLQGGSAGSPAR, DAPQDFHPDR, GAGTGGLGLAVEGPSEAK, YGGDELPFSPYR, YGGQPVPNFPSK	P21333
BCMP-1257	196.9	QQQTLQLQEESK	Q92817

Table 2 BCMP #	MW (kDa)	Amino Acid Sequences of Tryptic Digest Peptides	Accession Nos. of Identified Sequences
BCMP-1258	7.4-354.7	LPLENLQLLR, ELLLEFSK, TDLHAFENLELLR, VAPQSSEFLGA, GSTAENAEYLR, LPLENLQLLR, LTQLGTFEDHFLSLQR, NLQELLHGAVR, TDLHAFENLELLR, WMALESLLHR, YLVLQGDER, MHLPSPTDSNFYR, NYVVTDHGSCVR	P00533
BCMP-1259	76.2	STPAEDDSPGDSQVK	Q9UHB5
BCMP-1260	74.8	SNTENLSQHFR	Q9UHB6
BCMP-1261	30.1-85.2	EPLLPPEDDTR, GLDARPEVTR, NTGVLSVVTTGLDR, DTANWLELNPDTGALSTR, MALEVGDYK	P12830
BCMP-1262	40.3-40.8	FSTWTNTEFR	P07099
BCMP-1263	52.1-52.9	NSDPWAASQQPASSAGK	O95208
BCMP-1264	57.3	SSQAQAQELETK	Q14542
BCMP-1265	44.4	ELSDFLSYLQR	AAC51518
BCMP-1266	127.1	ELVSEFSR, MHLPSPTDSNFYR, TDLHAFENLELLR, VLGSGAFGTVYK, WMALESLLHR	Q15303
BCMP-1267	44.1-45.7	QLDMLLDEQR, YQEEFEHFQQELDK, AAFENWEVEVTFR,	P49257
BCMP-1268	33.6-35.0	FQDLGAAYEVLSDSEK, LALQLHPDR, TLEVELEPGVR	Q9UBS4
BCMP-1269	49.2	LGAVDESLSEETQK	Q9P1Q9
BCMP-1270	42.9	TALALALAQELGSK	Q9Y265
BCMP-1271	31.2-62.3	AVANYDSVEEGEK, LQSTFVFEELGR	P51659
BCMP-1272	30.5	YATDLLSVALNR	P56937
BCMP-1273	40.3-41.5	DYLEDQQGGR, EFWPQEVWSR, MGLGMAEFLDK	P37268
BCMP-1274	165.2	LSDSYSNTLPVR, TLMEQFNPSLR	O43858
BCMP-1275	82.7-248.1	DNLEFFLAGLGR, EDGLAQQQTQLNLR, EQGVTFPSGDLQEQLLR, FDASFFGVHPK	P49327

Table 2 BCMP #	MW (kDa)	Amino Acid Sequences of Tryptic Digest Peptides	Accession Nos. of Identified Sequences
BCMP-1276	219.2	YGGDELPYSPFR	Q9NS12
BCMP-1277	40.2-45.7	SPPVMVAGGR, VQVQVVER, AQADLAYQLQVAK, SQLLMQAEAEAASVR, AAYDLEVNTR	O75955
BCMP-1278	39.9-40.8	QLAVEAQELLR, SLLGTLTVEQLYQDR, VDYLSSLGK, TAEAQLAYELQGAR	Q14254
BCMP-1279	56.3	ELSEGDEVEVYSR	P51114
BCMP-1280	32.1	GLLAADESTGSLAK, LQSLGTENTEENR	P04075
BCMP-1281	37.2-40.2	LSGSVQLYCVHS	Q9H259
BCMP-1282	25.0-27.6	GNDVAFHFNPR, LQVLVEPDHFK, VAVNDAHLLQYNHR, LDNNWGR	P17931
BCMP-1283	30.3-30.7	NSFLQESWGEEER, ADVAFHFNPR, DLALHLNPR, EELTYDTPFK	O00214
BCMP-1284	38.3-39.5	LSGSVQLYCVHS	Q9NZ02-
BCMP-1285	24.8	YGGDELPYSPFR	O95303
BCMP-1286	44.4	ELSVLLLEMK, LQAALLSR	Q14789
BCMP-1287	71.2	SLAASSSFYGQR	S66258
BCMP-1288	55.4-61.5	AQQEQELAADAFK, LLLEDWK, ETMVTSTTEPSR, LWEEQLAAAK	Q9P0W9
BCMP-1289	72.5-78.7	EPWLLPSQHNDLLR, GLLEFEHQR, MMDYLQGSGETPQTDVR, VPDVLVADPPLAR	Q14697
BCMP-1290	33.1-34.4	LVLNGNPLTLFQER, GALQNLLPASTGAAK, LLSWYDNEFGYSNR, VVDLMAHMASK	P04406
BCMP-1291	25.2-27.8	FSLFAGGMLR, QLVEQVEQLQK, LYFHLGETEK, QLLDQVEQLQK	Q14437
BCMP-1292	105.9	LLELQELVLR	Q9NYF9
BCMP-1293	53.7	WGFGLEELYGLALR	Q9H3P7
BCMP-1294	31.4	LVPGPVFGSK	Q12907
BCMP-1295	34.4	LAQSDYLPTQQDVLR, LSQSNYLPTQQDVLR, MFDVGGQR	AAA35939
BCMP-1296	35.6	LAQSDYLPTQQDVLR	Q93020

Table 2 BCMP #	MW (kDa)	Amino Acid Sequences of Tryptic Digest Peptides	Accession Nos. of Identified Sequences
BCMP-1297	35.6	LLLLGAGESGK, LTESLNLFETLVNNR	AAH02722
BCMP-1298	34.7-35.9	LAQSDYLPTQQDVLR	P04899
BCMP-1299	29.3-31.6	LLLWDSYTTNK, LYAMHWGTDSR	P04901
BCMP-1300	31.2-36.6	LSQSNYLPTQQDVLR	P08754
BCMP-1301	72.5	LGAADYQPTEQDLLR	P09471
BCMP-1302	37.3	LATLGYLPTQQDVLR, MFVDLNPDSDK	P50148
BCMP-1303	36.6-37.2	LEDYFPEFAR	Q14433
BCMP-1304	36.6-36.9	LGEPDYLPSQQDLLLAR, VFLQYLPALR	Q14344
BCMP-1305	26.7-27.0	DGVVELTGK, LFDQAFGLPR	P04792
BCMP-1306	56.3-57.3	AQLHDLVLVGGSTR, GDTHLGGEDFDNR, ATAGDTHLGGEDFDNR, LLNEPTAAALAYGLDR, LVNHFVEEFK, NALESYAFNMK	P08107
BCMP-1307	69.6	LGLHEDSQNR	P07900
BCMP-1308	25.0-69.2	APFDLFENK, HFSVEGQLEFR, NPDDLTNEEYGEFYK, NPDDLTQEEYGEFYK, RAPFDLFENK, RAPFDLFENR	P08238
BCMP-1309	55.4	FEELNADLFR	P54652
BCMP-1310	31.9-33.6	YMGDLSGGQVLK, ETLEDGFPVHDGK	P30519
BCMP-1311	31.9-32.3	GGGGNFGPGPGSNFR	P22626
BCMP-1312	31.6-31.8	LFLGGLSFETTDESLR	S02061
BCMP-1313	85.8-94.6	NFLLDQTNVSAAAQR, SSGPTSLFAVTVAPPGAR	Q00839
BCMP-1314	94.6	LPEGPPNFLR, SQVNALEGELEEQR	O75146
BCMP-1315	29.5	LVDAVLGAELR, MGDADYLAAWHR	Q9UBN7
BCMP-1316	44.1-67.6	GVVDSEDLPLNLSR, NPDDLTQEEYGEFYK	AAC25497
BCMP-1317	29.1	AMEMLLQAAASGQGK	Q9UGJ2
BCMP-1318	28.0	ELETQTELLSPGSGR	AAF82399
BCMP-1319	248.1	QVTVQEGPLYR	XP_018332
BCMP-1320	45.0-179.3	LSDSYSNTLPVR, EGDLLTLLVPEAR	Q9UQB8

Table 2 BCMP #	MW (kDa)	Amino Acid Sequences of Tryptic Digest Peptides	Accession Nos. of Identified Sequences
BCMP-1321	71.2-73.0	AENGPGPGVLVLR	P08069
BCMP-1322	105.9-127.8	TQVGLLQYANNPR, GNWLLVGSPWSGFPENR, QAFDQLLQDR, SHLQYFGR	P17301
BCMP-1323	94.0	LEFDNDADPTSESK, LETTSNQDNLAPLTAK	P23229
BCMP-1324	7.4	STGLNAVPSQLLEGQWAAR	P06756
BCMP-1325	7.4-94.6	SLGTDLMNEMR, WDTGENPLYK	P05556
BCMP-1326	153.4-165.2	HVTQEFVSR, SEHSHSTTLPR, GMVEFQEGVELVDVR, DVVSFEQPEFSVSR	P16144
BCMP-1327	69.2-70.8	DEVLTWVDTLVK	P18084
BCMP-1328	67.6-76.2	ASVSVTAEDEGTQR, DGTFPLPLGESVTVTR, EPAVGEPAEVTTTVLVR, VTLNGVPAQPLGPR, TFLTVYWTPER, KELLLPGNNR	P05362
BCMP-1329	49.2	LMQSQLVSFYFK	P01579
BCMP-1330	29.6-67.6	LLNQPNQWPLVK, TMQNTSDLDTAR, ALMGSPQLVAAVVR, HPEAEMAQNSVR	P14923
BCMP-1331	33.1-33.3	LTASYEDR, NGYGTPMTSNAVR	Q9Y624
BCMP-1332	291.0	AWDDFFPGSDR	O75915
BCMP-1333	39.9-41.2	GPLPAAPPVAPER	O94962
BCMP-1334	121.3-127.8	LQQEEVQK, QEALPLHQETK, QTEDSLASER, SVLAETEGLLQK, EASSASQFEELELVLK, ELSGLWNELDSLK, TQLLQDVQDENK	S32763
BCMP-1335	115.6-165.2	ELSGLWNELDSLK, SVEELLEAELLK	XP_007337
BCMP-1336	31.2	DSPLQCLQALAENR	P02788
BCMP-1337	60.4	NPDGGFATYETK	P48449
BCMP-1338	32.3-105.9	SADGSAPAGEGEGVTLQR	Q01650
BCMP-1339	52.1	LVLTNNQLTTLPR	O76063
BCMP-1340	61.1	QAELEELYESSLR	P48960

Table 2 BCMP #	MW (kDa)	Amino Acid Sequences of Tryptic Digest Peptides	Accession Nos. of Identified Sequences
BCMP-1341	31.0-32.5	EPAAPVSLQR, GFSVVADTPELQR, QQSELQSQVR, MGPSGGEGMEPER, YHEEFEK	Q14847
BCMP-1342	26.0-26.2	EQNSPLYLSR, TEEGLGFNLMGGK	Q9HAP6
BCMP-1343	30.3-32.5	TLHPDLGTDK, DLADELALVDVLEDK	P00338
BCMP-1344	61.5	ADFFEDENGQR	XP_002405
BCMP-1345	59.3-69.2	VEDYDAADDVQLSK, LSWSQLGGSPAEPLPGR, LSVPPLVEVMR, LVLAEAQVGDER, SPPYQLDSQGR, GDGSPSPEYTLFR, VAYLDPLELSEGK	P50895
BCMP-1346	88.5	AGYLLPLQGPGLTTTESR, RYEVPLETPR	P10253
BCMP-1347	66.7	LPLNDLFR	Q9UD93
BCMP-1348	59.3-71.2	GLLTVDELLALR, LPLNDLFR	P13473
BCMP-1349	29.5-35.6	APEFSMQGLK, TQNDVDLADVAYYFEK	P16422
BCMP-1350	32.8	FVEGLPLNDFSR	P40925
BCMP-1351	69.6	FLSGHTSELGDFR	XP_002747
BCMP-1352	25.6-26.2	FYGPEGPYGVFAGR	O00264
BCMP-1353	34.4	QVGGQAVTGDGVNDSPALK	Q13817
BCMP-1354	76.2	DALNENSQLQESQK	O15320
BCMP-1355	35.9-40.3	DGEDQTQDTELVETR, WAAVVVPSGEEQR, FDSDAASPR, FLSVGYVDDTQFVR, APWVEQEGPEYWDR, DYLALNEDLR, FLAVGYVDDTQFVR, SWTAADMAAQLTQR	O19589
BCMP-1356	35.6	APWVEQEGPEYWDR, FLSVGYVDDTQFVR	Q9TNT4
BCMP-1357	61.1-64.0	ALQLEEER, APDFVFYAPR, ELSEQLQR, LALLEEAR, LGFPWSELR, QLLTLSSELSQAR, EDEVEEWQHR, ELHKPGYLANDR, QLFDQVVK, THNDLLHNENMR, SQEQLAAELAEYTAK	P26038

Table 2 BCMP #	MW (kDa)	Amino Acid Sequences of Tryptic Digest Peptides	Accession Nos. of Identified Sequences
BCMP-1358	38.7	EPQPEVAAAEEEK	O15427
BCMP-1359	120.7	EPEVEEEEEKPR	Q92794
BCMP-1360	7.4-249.0	GPVGTVSEAQLAR, SLSQLHEAAVR, LYPLPDDPSVPAPPR, SLGPPGPPFNLTPR, VSMFVLGTGDEPPPER, EELYMPPLVLK, LEGALGADTTEDGDEK, VEMTLELLNEK, LDVGFVYDEPGHAVMR, LEGALGADTTEDGDEK, LLWHPVMNGDK, QLSGNNLRPVVK, EYTGFPDPYDELNTGK, , LLVELATFLEK	Q9NZM1
BCMP-1361	7.4	STQGVTLTDLQEAEK	O14974
BCMP-1362	52.9	ELWFFLQSELK, LPEGPLDQGPALGR	O00235
BCMP-1363	34.1	DPQLVPLLLEAAR, LLLTSSASVLFEGVDLK	Q15738
BCMP-1364	29.1	EGHLSPDLVAEQK	P15559
BCMP-1365	29.7-32.8	DLLLRPELEELR, STPALTLESPDLK, GPSGLLVYQGK	O75675
BCMP-1366	87.9-90.8	LVVENVDVLTQMR, EYLTSHLELR	Q9Y2A7
BCMP-1367	32.8	YSSPVDMLGLVLAK	Q15346
BCMP-1368	7.4-249.0	AEGPEVDVNLPK, GPEVDVNLPK, LSMPDVDLNLK, LSMSEVDLNVAAPK, VDVDVPDVNLEGPDAK, LPSGSGAASPTGSAVDLR, VNVEAPDVNLEGLGGK, APDVDVNLAGPDAALK, GEGPEVDMNLPK, GGVTGSPEASLSGSK	Q09666
BCMP-1369	49.9-153.4	EVLDSFLDLAR, NLFPSNLVSAAFR, SYSTTYEER, LVEMEDVGLLFAR, GPAGDATVASEK	Q15758

Table 2 BCMP #	MW (kDa)	Amino Acid Sequences of Tryptic Digest Peptides	Accession Nos. of Identified Sequences
BCMP-1370	44.0	ENDHLFLSLTEQR	Q13137
BCMP-1371	47.3-49.2	ELDFVSHHVR, ELQQAVLHMEQR, EVWEELDGLDPNR, LVTLEEFLASTQR, YLESLGEEQR	Q02818
BCMP-1372	41.5-42.9	LEPPDTGLYYDEYLK, QVLDVLETDK, EYENLLALQENELK, ELDLVSHHVR	P80303
BCMP-1373	59.3-76.7	FGYVDFESAEDLEK, GYAFLEFASFEDAK	P19338
BCMP-1374	33.1-34.7	VDNDENEHQLSLR	P06748
BCMP-1375	121.3	ANLLNNLFELAGLGK	Q9UIQ6
BCMP-1376	143.6	ELFLQQER, ELSSYEDFLDAR, LPTAVVPLR, YFAATQFEPLAAR	Q9UIQ7
BCMP-1377	66.7-121.3	QDVYGPQPQVR, GYELLFQPEVVR	O60713
BCMP-1378	65.3	QDVYGPQPQVR	O60715
BCMP-1379	55.4-78.7	GYELLFQPEVVR, NFHYPPDGYSR, QDVYGPQPQVR, SLDNNYSTPNER, AAALVLQTLWGYK	O60716
BCMP-1380	24.2-24.8	TVLDSQTHYR, QYDSFTYR	Q9Y3Q3
BCMP-1381	105.9	SLLQGTLLQYVK	AAD45723
BCMP-1382	54.5-55.4	DFTSLENTVEER, LALQALTEK, LEGLGSSEADQDGLASTVR, LPPQDFLDR, TAVDSLVAYSVK	Q07065
BCMP-1383	43.9-44.6	ADSAVSQEQLR, ALYDYAGQEADELSFR, LSALHLEVR, AQYEQTLAELHR	Q9H331
BCMP-1384	34.4-45.2	AAGEVDLGDAAYYFER, THHLLLDLR	P09758
BCMP-1385	34.1-40.3	TVTATFGYPFR	Q16563
BCMP-1386	56.3	DLDSGSPAEEAGLK	O60450
BCMP-1387	153.4	LQLEEEQHR, VVLQQDPQQAR	O60437
BCMP-1388	24.2-25.5	QLTVNDLPVGR, QGGLGPMNLPLVSDPK	Q06830
BCMP-1389	27.2-27.6	QLTLNDLPVGR	Q13162
BCMP-1390	55.4	NPGFELLHGLLDR	Q9NSD9
BCMP-1391	165.2	VMEPLLQLLQQK	Q9UPG2
BCMP-1392	28.3	HGESAWNLENR	P18669

Table 2 BCMP #	MW (kDa)	Amino Acid Sequences of Tryptic Digest Peptides	Accession Nos. of Identified Sequences
BCMP-1393	62.7-71.2	ALMGSPQLVAAVVR, LLWTTSR, SPQMVSALVR	Q15151
BCMP-1394	249.0-472.2	AQVEQELTTLR, QLAEEDLAQQR, QVQVALETAQR, WQAVLAQTDVR, LQEAGLLSAEELQR	Q15149
BCMP-1395	105.9	GASSPLLTVFTDDK	Q15155
BCMP-1396	7.4	LASVPGSQTVVVK	O00592
BCMP-1397	164.4	LYYENLLLGVPR, TMSQLSSTLSR	Q9UNJ0
BCMP-1398	52.1-53.7	AAEVWMDEYK, WYLENVYPELR	Q10471
BCMP-1399	60.4-61.5	LAEVWMDEYK, TVVTGEQLWELQK	Q14435
BCMP-1400	57.3	SPAMAGGLFALER	XP_003527
BCMP-1401	29.6	NNFAVGYR	AAA60145
BCMP-1402	32.8	QDVDDEYGVSQALAR, TLQTLALLTYLMEHK	P51532
BCMP-1403	28.0	LLPQLTYLDGYDR	P39687
BCMP-1404	45.0-47.3	FLNEMLAPVMR, SDFYDLVLVATPLNR	Q9UHG3
BCMP-1405	85.8	SMENLMEMNK	Q12959
BCMP-1406	54.5-55.4	TAQEVETYR	P17844
BCMP-1407	62.7	QVGYEDQWLQLLR	O60568
BCMP-1408	28.7-28.9	LFTSLGEDYDER, FDAGELLTQR	P35232
BCMP-1409	66.7	DVGDESFQVGLLR	Q16549
BCMP-1410	41.1	EQAPLLPTPTVLDPSR	O15191
BCMP-1411	29.3	QNLFQEAEEFLYR	Q15129
BCMP-1412	42.4	LQEVLETLLSLEK	O00232
BCMP-1413	110.5-115.6	VEMQPTELVSK, GSQPPPAAESQSSLR	O43491
BCMP-1414	45.0-46.1	FVMQEEFSR, MDATANDVPSPYEVR, ELSDFLSYLQR, FLQDYFDGNLK, FVMQEEFSR, KQAGPASVPLR, LNFAVASR	P30101
BCMP-1415	52.1	MDATANDVPSDR	P13667

Table 2 BCMP #	MW (kDa)	Amino Acid Sequences of Tryptic Digest Peptides	Accession Nos. of Identified Sequences
BCMP-1416	41.5-45.7	NSYLEVLLK, GESPVDYDGGR, GSTAPVGGGAFPTLVER, LAAVDATVNQVLASR, NLEPEWAAAASEVK, TGEALVDAALSALR	Q15084
BCMP-1417	46.1-50.6	DHENLVLAK, LLTLEEEMTK, YKPESEELTAER, VDATEESDLAQQYGVR, YKPESEELTAER, HNQLPLVLEFTEQTAPK, LLEFFGLK, LLFLFLDSDHTDNQR	P07237
BCMP-1418	29.1	ESLQQMAEVTR	P14314
BCMP-1419	62.3	LLAEALNQVTQR	Q05655
BCMP-1420	45.2	LLESAEELLR	Q13217
BCMP-1421	41.5-43.6	QLELENLTTQETR, SGSWAALYQDLR, DVSPFDHSR, FLMGDSSVQDQWK	P18031
BCMP-1422	39.8-40.6	MQDTMEENSESALR	P17706
BCMP-1423	37.6	FQLLNNTEGDWWEAR, LLLNPGNQR	P07947
BCMP-1424	56.3-109.9	YEGVVDMFQTVK	P10586
BCMP-1425	76.7	TNVNVFSELSAPR	O95357
BCMP-1426	49.2-127.8	LDLDSPPLTAR, DPVQEAWAEDVDLR, EAEAALYHLQLFEELR, GADFLVTEVENGGSLGSK	P14618
BCMP-1427	25.1-26.6	LQLWDTAGQER, QWLQELDR, GAAGALMVYDLTR	Q9H0Z8
BCMP-1428	109.9-153.4	GLQQQNSDWYLK, LFYPETTDLYDR, LQQTYAALNSK, TLLNAEDPPMVVVR, VDQLQELVTGNPTVLK, ALQSPALGLR, ATFYGEQVDYYK, LGLAPQLQDLYGK, EELQSSLSGVTAAYNR, EQLSDMMMLNK, FDVPGDENAEMDAR, QSGQTDPLQK, RLPADTFAALK	P46940

Table 2 BCMP #	MW (kDa)	Amino Acid Sequences of Tryptic Digest Peptides	Accession Nos. of Identified Sequences
BCMP-1429	143.6	VDFTEEELSNMR	Q13576
BCMP-1430	24.2-24.8	NLDEHANEDVER, AFLTLAEDLLR, FHTLTTSYYR	O88386
BCMP-1431	25.7-26.0	GAAGALMVYDLTR	P35287
BCMP-1432	24.3	LALWDTAGQER	Q9NP72
BCMP-1433	24.2-25.0	GAAGALLVYDLTR, KEEGEAFAR, LQLWDTAGQESFR, LQEGVFDLNNEANGLK, TASNVEEAFLNTAK, DTFNHLTTWLEDAR	P08886
BCMP-1434	25.2-25.5	TTLGVEFSTR, HQTYAVVER	P57735
BCMP-1435	24.8-26.0	QASPSLVLALAGNK, FELWDTAGQER, GVDLQENNPASR, SEPQNLGGAAGR	P35239
BCMP-1436	25.1-26.1	GVDLQENNPASR, QASPNLVLALAGNK	P51148
BCMP-1437	24.4-25.1	GSDVLLMLVGNK, VAAALPGMESTQDR, ELNVMFLETSAK	P20340
BCMP-1438	25.7	LEGNSPQGSNQGVK, NLEEHASADVEK	P24407
BCMP-1439	25.4-26.2	AEQWNVNYVETSAK	P11233
BCMP-1440	24.3	LNVDEAFEQLVR	P10301
BCMP-1441	24.2-24.3	QVTQEEGQQLAR	P17082
BCMP-1442	120.7	GLQSLPTHDPSPLQR	P04626
BCMP-1443	196.9-219.2	LGQLYQSWLDK	O15258
BCMP-1444	65.3-66.7	AQVDDLTEAVAAK	Q03962
BCMP-1445	35.6-37.9	EQFNEFR, YLFDNVAK, ELVVLETLEDLDK	Q15293
BCMP-1446	41.1	GPLPAAPPVAPER, SDEGHPFR	AAH07109
BCMP-1447	25.0-26.4	TQLDHYVGLAR	O95197
BCMP-1448	41.1	AVLSAEQLR, LYGPSSVSFADDFVR	Q9NPA9
BCMP-1449	27.0-27.6	LATQLTGPVMPVR, STESLQTNVQR	O60250
BCMP-1450	29.1-29.6	VPPALNQFTQALDR	AAH05128
BCMP-1451	94.6	ESTLHLVLR	AAH05328
BCMP-1452	24.3	HLDFSLR, QVVNLPSFLVR	AAA85659
BCMP-1453	32.1-33.1	GHLENNPALEK, LLQLLDDYPK	XP_007036
BCMP-1454	63.5-65.3	GDATVSYEDPPTAK	Q01844

Table 2 BCMP #	MW (kDa)	Amino Acid Sequences of Tryptic Digest Peptides	Accession Nos. of Identified Sequences
BCMP-1455	74.8-472.2	LGLFGQDEDVTSK, DLVPGDLVELAVGDK, VDQSLLTGESVSVLK, EFDELNPSAQR	P16615
BCMP-1456	71.2-73.0	QMQEAEMMYQTGMK	Q9UBV2
BCMP-1457	71.2	AYAALTDEESR	Q9UGP8
BCMP-1458	29.7-30.1	EHALAQAELLK, DPSVTQVTR, NVPPGLDEYNPFSDSR	O15126
BCMP-1459	31.6-32.8	ELQNTVANLHVR	O15127
BCMP-1460	30.5	AQQEFAAGVFSNPAVR, ELQHAALGGTATR, TAAANAAAGAAENAFR	O14828
BCMP-1461	45.2-46.1	GNEAELYLDLGAYGEPR	Q9HBA8
BCMP-1462	45.7	DVTNNVHYENYR, FEDYLNAESR	Q16181
BCMP-1463	46.1-47.3	NLGVVVVGFPATPLLESR	O15270
BCMP-1464	62.7	GDADSVLSLTFR	Q9HCA3
BCMP-1465	31.0-31.1	NVQLQENELR, YPENFFLLR	P08129
BCMP-1466	30.3	NVQLQENELR, YPENFFLLR	P36873
BCMP-1467	37.6-472.2	AVFQANQENLPLLK, GQELPLDEQWR, VDNSSLTGESEPQTR, DGPNALTPPPTTPEWLK, LNLPVSQVNPR, NPNTSEPQHLLVMK, SPDFTNENPLETR, DAFQNAYLELGGLGER	P05023

Table 2 BCMP #	MW (kDa)	Amino Acid Sequences of Tryptic Digest Peptides	Accession Nos. of Identified Sequences
BCMP-1468	7.4-355.7	AVFQANQENLPLLK, DAFQNAYLELGGLGER, GLVVYTGDR, GVGLLSEGNETVEDLAAR, MSLNAEEVVVGDLVEVK, NMVPQQALVLR, SPDFTNENPLETR, VDNSSLTGESEPQTR, QGALVAVTGDGVNDSPALK, LNLPVSQVNPR, YHTELVFAR, NMVPQQALVLR, VLMVTGDHPLTAK, YEPAAVSEQGDK	P50993
BCMP-1469	120.7	DAFQNAYLELGGLGER, LNLPVSQVNPR	P13637
BCMP-1470	43.1	VAPPGLTQLPQLQK	P05026
BCMP-1471	32.3-38.7	LFLYNPTTGEFLGR, LLGLKPEGVPR	P54709
BCMP-1472	55.4-60.4	LGPEGELLLR, NLFPSNLVSAAFR, SYSTTYEER	Q9UNP2
BCMP-1473	36.3-36.6	LENYPLPEPGPNEVLLR	Q00796
BCMP-1474	69.6-76.7	DPLYFTGLASEPGAR, FLFASVMADK, TEFGMALGPENSGK	Q99523
BCMP-1475	179.3-291.0	ESAPGLLLATGSVGK, NLLVNTLYTVR, TNLYLSEAEGLK, ENQEVLLEEVR	Q92673
BCMP-1476	49.2	AGYPLEHPFDFR	O95470
BCMP-1477	72.5	FGQGGAGPVGGQGPR	P23246
BCMP-1478	62.3-64.8	VDQSAVGFEYQGK	Q14247
BCMP-1479	26.9-27.3	GLGAGAGAGEESPATSLPR, DFSLEQLR,	O15173
BCMP-1480	31.1	GTEDFLVESLDASFR	P43307
BCMP-1481	29.5-30.3	MTQLVLPGMVER, TLAVDFASEDLYDK	Q9Y6G8
BCMP-1482	71.2-78.7	SQDVELWEGEVVK	Q9P0H8
BCMP-1483	28.0	QQYLQSLEER	P21912
BCMP-1484	143.6-355.7	NLALGGGLLLLLAESR, MWFQWSEQR	O15260

Table 2 BCMP #	MW (kDa)	Amino Acid Sequences of Tryptic Digest Peptides	Accession Nos. of Identified Sequences
BCMP-1485	26.4	LEEGLDQLNK	O00161
BCMP-1486	29.5	GAGSAMSTDAYPK, KLDDTDPVPR	O95721
BCMP-1487	29.4-29.9	SPSDLLDASAVSATSR, EELDWTTNELR, WTELLQDPSTATR	O60499
BCMP-1488	33.6-35.0	WVDGVDELQYDVGR, QLVQSLSDLNELFR	O14662
BCMP-1489	34.1-34.4	QLVQSLSDLNELFR	Q9H1T7
BCMP-1490	32.1	HLEEDEVR	Q13277
BCMP-1491	53.7	MTDLLEEGLTVVENLYK	Q9UPD7
BCMP-1492	31.4	QLAQLQDFVR	Q13190
BCMP-1493	32.1-33.1	EFGSLPTTPSEQR, TLNQLGTPQDSPELR, QQYTNQLAK	O15400
BCMP-1494	52.9	MTDLLEEGLTVVENLYK, THSQLLLLDR	O00186
BCMP-1495	32.8	SVVYFALLTCCLLGR	P03986
BCMP-1496	47.9	GDVTLTNDGATLLK	P50991
BCMP-1497	28.4-28.9	VDVTEQPGLSGR	Q9H3N1
BCMP-1498	47.3	SELVANNVTLPAGEQR, SSTPLPTLSSSAENTR	P42166
BCMP-1499	46.1	SSTPLPTLSSSAENTR	Q16295
BCMP-1500	29.7	LNLVGGMFDTVQR	AAD22033
BCMP-1501	121.3	SNPSAVAGNETPGASTK, SSEPVQHEESLR	Q9UDY2
BCMP-1502	90.8-94.6	AVGVGPGSSAGSNAR, HLTDSGLAAR, SSEPVQADESQSPR	O95049
BCMP-1503	31.4	GYELDEDLVSR	O14656
BCMP-1504	31.9	VAEEMTFFPR	O14657
BCMP-1505	40.6	APVLDLGLANTGK	Q9Y4P3
BCMP-1506	26.9-249.0	SAFSNLFGGEPLSYTR, AAAEVAGQFVLK, VEYHFLSPYVSPK, VSASPLLYTLLEK, EEPGEDFPAAR, RLYWDDLK, LYWDDLK, YNSQLLSFVR, LAVDEEENADNNTK, DENLALYVENQFR, LTTDFGNAEK, SSGLPNLPVQTLSR, QVDGDNSHVEMK	P02786

Table 2 BCMP #	MW (kDa)	Amino Acid Sequences of Tryptic Digest Peptides	Accession Nos. of Identified Sequences
BCMP-1507	24.4	NVLGLQMGTNR	P37802
BCMP-1508	66.6-70.8	VSASPLLYTLLEK, SGAEEQGPLDGPSK, EAEDDDTGPEEGSPPK	O43493
BCMP-1509	73.0	ESLESELR, KGDLFLVR	P55072
BCMP-1510	82.7	VDLGGFAGLFDLK	P22102
BCMP-1511	35.6	LLLDMSVSFHTHVK	AAC34245
BCMP-1512	29.7	LQLVEEELDR, LQVLQQQADDAEER	Q9NQH8
BCMP-1513	36.9	HAEVVASLK, LLEVNGQNVEGLR, VEPGSPAEAAALR	Q15599
BCMP-1514	50.6	LFGGFNSSDTVTSPQR	P12931
BCMP-1515	94.0	HGLPLEEVAK	P29597
BCMP-1516	24.2	ELEQEAAVELSQLR	P47985
BCMP-1517	60.4	DEEWELAQDQLLR	Q9UIV5
BCMP-1518	127.1-127.8	LLETTTFFQR, LEYQFFEDR, VEHVVSVLEK	Q9NYU2
BCMP-1519	354.7-472.2	AFEADSTVLEK, QLGEVAAFGGSNLEPSVR, LLEEYGSDDTR, VFLADQPLEAPEEPR	P46939
BCMP-1520	30.5-30.9	EPLLPPEDDTR, GLDARPEVTR	Q15855
BCMP-1521	46.1	AVVQVFEGTSGLDAK, LPQSTLSEFYPR	P21281
BCMP-1522	30.7-34.4	LLFEGAGSNPGDK, FFEHEVK, LYPEGLAQLAR, TLAYLLTELDER	Q02547
BCMP-1523	30.7	TLAYLLTELDER	Q9Y5K8
BCMP-1524	29.4	LDLLAQQMMPEVR	P36543
BCMP-1525	52.9-55.4	HVTVVGELSR, SFLEEVLASGLHSR, TEVPPLLLLLDR	Q15715
BCMP-1526	28.4-47.3	VEQVLSLEPQHELK, TVQSNSPLSALAPTGK	Q9P0H0
BCMP-1527	28.3-28.7	VEQVLSLEPQHELK	O95292
BCMP-1528	29.1	VEQVLSLEPQHELK	O95293
BCMP-1529	28.1-30.5	GPFTDVVTTNLK	O75453
BCMP-1530	25.5-27.8	EQNSPLYLSR	O14910

Table 2 BCMP #	MW (kDa)	Amino Acid Sequences of Tryptic Digest Peptides	Accession Nos. of Identified Sequences
BCMP-1531	25.4-26.2	NLGSLNTELQDVQR, DLQQYQSQAK, LMVANLEEVLQR, VADGLPLAASMQEDEQSGR	O75396
BCMP-1532	26.9-27.0	AMLLQGTESLNR, GLHEDLQGVPER	Q9UEU0
BCMP-1533	27.1-27.2	GLHEDLQGVPER	AAH03142
BCMP-1534	61.5-64.0	DFQSGQHVLVR, EFSDLFR, YVGESEANLR	P46459
BCMP-1535	31.8	LENSTFFHK	P78511
BCMP-1536	29.8	NNFAVGYR	XP_005893
BCMP-1537	25.6	GVDLTEPTQPTR	P20339
BCMP-1538	25.0	HLTYENVER, GAVGALLVYDIAK, AQIWDTAGQER, STIGVEFATR	Q15907
BCMP-1539	50.5	WPFSLSEQQLDAR	AAK97797
BCMP-1540	39.8	LTGPAAAEPR	Q9BX05
BCMP-1541	67.6	EGNELQFIQLVK	Q9BZ74
BCMP-1542	105.9	SLLQGTILQYVK	Q9UN29
BCMP-1543	53.6	LMVPVTVVFTR	Q96S52
BCMP-1544	29.0	VADGGGAGGTFQPYLDTLR, AIVFVVDSAAFQR	Q9Y5M8
BCMP-1545	28.2	AYSEAHEISK	P35214

For any given BCMP, the detected level obtained upon analyzing breast tissue from subjects having breast cancer relative to the detected level obtained upon analyzing breast tissue from subjects free from breast cancer will depend upon the particular analytical protocol and detection technique that is used, provided that such BCMP is differentially expressed between normal and disease tissue. Accordingly, the present invention contemplates that each laboratory will establish a reference range for each BCMP in subjects free from breast cancer according to the analytical protocol and detection technique in use, as is conventional in the diagnostic art. Preferably, at least one control positive breast tissue sample from a subject known to have breast cancer or at least one control negative breast tissue sample from a subject known to be free from breast cancer (and more preferably both positive and negative control samples) are included in each batch of test samples analysed.

In one embodiment, the level of expression of a protein is determined relative to a background value, which is defined as the level of signal obtained from a proximal region of the image that (a) is equivalent in area to the particular feature in question; and (b) contains no discernable protein feature.

BCMPs can be used for detection, prognosis, diagnosis, or monitoring of breast cancer or for drug development. In one embodiment of the invention, breast tissue from a subject (*e.g.* a subject suspected of having breast cancer) is analysed by 1D-electrophoresis for detection of one or more of

the BCMPs. A change in the abundance of said one or more BCMPs in the breast tissue from the subject relative to breast tissue from a subject or subjects free from breast cancer (*e.g.* a control sample) or a previously determined reference range indicates the presence or absence of breast cancer.

In a preferred embodiment, breast tissue from a subject is analysed for quantitative detection of clusters of BCMPs.

As will be evident to one of skill in the art, a given BCMP can be described according to the data provided for that BCMP in Tables 1-2. The BCMP is a protein comprising a peptide sequence described for that BCMP (preferably comprising a plurality of, more preferably all of, the peptide sequences described for that BCMP).

In one embodiment, breast tissue from a subject is analysed for quantitative detection of one or more of the BCMPs or any combination of them, wherein a change in abundance of the BCMP or BCMPs (or any combination of them) in the breast tissue from the subject relative to breast tissue from a subject or subjects free from breast cancer (*e.g.* a control sample or a previously determined reference range) indicates the presence of breast cancer.

As shown above, the BCMPs described herein include previously undescribed proteins, as well as known proteins not previously associated with breast cancer. For each BCMP the present invention additionally provides: (a) a preparation comprising the isolated BCMP; (b) a preparation comprising one or more fragments of the BCMP; and (c) antibodies that bind to said BCMP, to said fragments, or both to said BCMP and to said fragments. As used herein, a BCMP is "isolated" when it is present in a preparation that is substantially free of contaminating proteins, *i.e.*, a preparation in which less than 10% (preferably less than 5%, more preferably less than 1%) of the total protein present is contaminating protein(s). A contaminating protein is a protein having a significantly different amino acid sequence from that of the isolated BCMP, as determined by mass spectral analysis. As used herein, a "significantly different" sequence is one that permits the contaminating protein to be resolved from the BCMP by mass spectral analysis, performed according to the Reference Protocol.

The BCMPs of the invention can be assayed by any method known to those skilled in the art, including but not limited to, 1D-gel electrophoresis described herein, kinase assays, enzyme assays, binding assays and other functional assays, immunoassays, and western blotting. In one embodiment, the BCMPs are separated on a 1-D gel by virtue of their MWs and visualized by staining the gel. In one embodiment, the BCMPs are stained with a fluorescent dye and imaged with a fluorescence scanner. Sypro Red (Molecular Probes, Inc., Eugene, Oregon) is a suitable dye for this purpose. A preferred fluorescent dye is disclosed in US Patent No. 6,35,446.

Alternatively, BCMPs can be detected in an immunoassay. In one embodiment, an immunoassay is performed by contacting a sample from a subject to be tested with an anti-BCMP antibody under conditions such that immunospecific binding can occur if the BCMP is present, and detecting or measuring the amount of any immunospecific binding by the antibody. Anti-BCMP antibodies can be produced by the methods and techniques taught herein.

In one embodiment, binding of antibody in tissue sections can be used to detect aberrant BCMP localization or an aberrant level of one or more BCMPs. In a specific embodiment, antibody to a BCMP can be used to assay a patient tissue (*e.g.* a breast tissue biopsy) for the level of the BCMP

where an aberrant level of BCMP is indicative of breast cancer. As used herein, an "aberrant level" means a level that is increased or decreased compared with the level in a subject free from breast cancer or a reference level. If desired, the comparison can be performed with a matched sample from the same subject, taken from a portion of the body not affected by breast cancer.

Any suitable immunoassay can be used, including, without limitation, competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays and protein A immunoassays.

For example, a BCMP can be detected in a fluid sample (e.g. blood, urine, or breast tissue homogenate) by means of a two-step sandwich assay. In the first step, a capture reagent (e.g. an anti-BCMP antibody) is used to capture the BCMP. The capture reagent can optionally be immobilized on a solid phase. In the second step, a directly or indirectly labeled detection reagent is used to detect the captured BCMP. In one embodiment, the detection reagent is a lectin. Any lectin can be used for this purpose that preferentially binds to the BCMP rather than to other isoforms that have the same core protein as the BCMP or to other proteins that share the antigenic determinant recognized by the antibody. In a preferred embodiment, the chosen lectin binds to the BCMP with at least 2-fold greater affinity, more preferably at least 5-fold greater affinity, still more preferably at least 10-fold greater affinity, than to said other isoforms that have the same core protein as the BCMP or to said other proteins that share the antigenic determinant recognized by the antibody. Based on the present description, a lectin that is suitable for detecting a given BCMP can readily be identified by methods well known in the art, for instance upon testing one or more lectins enumerated in Table I on pages 158-159 of Sumar *et al.*, Lectins as Indicators of Disease-Associated Glycoforms, *In*: Gabius H-J & Gabius S (eds.), 1993, Lectins and Glycobiology, at pp. 158-174. In an alternative embodiment, the detection reagent is an antibody, e.g. an antibody that immunospecifically detects other post-translational modifications, such as an antibody that immunospecifically binds to phosphorylated amino acids. Examples of such antibodies include those that bind to phosphotyrosine (BD Transduction Laboratories, catalog nos.: P11230-050/P11230-150; P11120; P38820; P39020), those that bind to phosphoserine (Zymed Laboratories Inc., South San Francisco, CA, catalog no. 61-8100) and those that bind to phosphothreonine (Zymed Laboratories Inc., San Francisco, CA, catalogue nos. 71-8200, 13-9200).

If desired, a gene encoding a BCMP, a related gene, or related nucleic acid sequences or subsequences, including complementary sequences, can also be used in hybridization assays. A nucleotide encoding a BCMP, or subsequences thereof comprising at least 8 nucleotides, preferably at least 12 nucleotides, and most preferably at least 15 nucleotides can be used as a hybridization probe. Hybridization assays can be used for detection, prognosis, diagnosis, or monitoring of conditions, disorders, or disease states, associated with aberrant expression of genes encoding BCMPs, or for differential diagnosis of subjects with signs or symptoms suggestive of breast cancer. In particular, such a hybridization assay can be carried out by a method comprising contacting a subject's sample containing nucleic acid with a nucleic acid probe capable of hybridizing to a DNA or RNA that

encodes a BCMP, under conditions such that hybridization can occur, and detecting or measuring any resulting hybridization. Nucleotides can be used for therapy of subjects having breast cancer, as described below.

The invention also provides diagnostic kits, comprising an anti-BCMP antibody. In addition, such a kit may optionally comprise one or more of the following: (1) instructions for using the anti-BCMP antibody for diagnosis, prognosis, therapeutic monitoring or any combination of these applications; (2) a labeled binding partner to the antibody; (3) a solid phase (such as a reagent strip) upon which the anti-BCMP antibody is immobilized; and (4) a label or insert indicating regulatory approval for diagnostic, prognostic or therapeutic use or any combination thereof. If no labeled binding partner to the antibody is provided, the anti-BCMP antibody itself can be labeled with a detectable marker, *e.g.* a chemiluminescent, enzymatic, fluorescent, or radioactive moiety.

The invention also provides a kit comprising a nucleic acid probe capable of hybridizing to RNA encoding a BCMP. In a specific embodiment, a kit comprises in one or more containers a pair of primers (*e.g.* each in the size range of 6-30 nucleotides, more preferably 10-30 nucleotides and still more preferably 10-20 nucleotides) that under appropriate reaction conditions can prime amplification of at least a portion of a nucleic acid encoding a BCMP, such as by polymerase chain reaction (see, *e.g.* Innis *et al.*, 1990, PCR Protocols, Academic Press, Inc., San Diego, CA), ligase chain reaction (see EP 320308) use of Qβ replicase, cyclic probe reaction, or other methods known in the art).

Kits are also provided which allow for the detection of a plurality of BCMPs or a plurality of nucleic acids each encoding a BCMP. A kit can, optionally, further comprise a predetermined amount of an isolated BCMP protein or a nucleic acid encoding a BCMP, *e.g.* for use as a standard or control.

Use in Clinical Studies

The diagnostic methods and compositions of the present invention can assist in monitoring a clinical study, *e.g.* to evaluate drugs for therapy of breast cancer. In one embodiment, candidate molecules are tested for their ability to restore BCMP levels in a subject having breast cancer to levels found in subjects free from breast cancer or, in a treated subject (*e.g.* after treatment with taxol or doxorubacin), to preserve BCMP levels at or near non-breast cancer values. The levels of one or more BCMPs can be assayed.

In another embodiment, the methods and compositions of the present invention are used to screen candidates for a clinical study to identify individuals having breast cancer; such individuals can then be excluded from the study or can be placed in a separate cohort for treatment or analysis.

Purification of BCMPs

In particular aspects, the invention provides isolated BCMPs, preferably human BCMPs, and fragments and derivatives thereof which comprise an antigenic determinant (*i.e.*, can be recognised by an antibody) or which are otherwise functionally active, as well as nucleic acid sequences encoding the foregoing. "Functionally active" as used herein refers to material displaying one or more functional activities associated with a full-length (wild-type) BCMP, *e.g.* binding to a BCMP substrate or BCMP binding partner, antigenicity (binding to an anti-target antibody), immunogenicity, enzymatic activity etc.

As used herein, the term "derivative" refers to a polypeptide that comprises an amino acid sequence of a parent polypeptide that has been altered by the introduction of amino acid residue substitutions, deletions or additions, and/or amino acid modifications such as but not limited to, phosphorylation and glycosylation. The derivative polypeptide preferably possesses a similar or identical function to the parent polypeptide. Such derivatives preferably exhibit the immunological or biological activity of the BCMPs of the invention. Alternatively, the biological activity of the BCMP may be altered. As such, it will be appreciated by one skilled in the art that derivatives can include post-translational modifications, for example but without limitation, phosphorylation and glycosylation.

In specific embodiments, the invention provides fragments of a BCMP comprising at least 1% of the length of a BCMP, at least 5%, at least 10%, at least 50%, at least 75%, or at least 95% of the length of a BCMP. Fragments lacking some or all of the regions of a BCMP are also provided, as are proteins (*e.g.* fusion proteins) comprising such fragments. Any given fragment of BCMP may or may not possess a functional activity of the parent polypeptide. Nucleic acids encoding the foregoing are provided.

Once a recombinant nucleic acid which encodes the BCMP, a portion of the BCMP, or a precursor of the BCMP is identified, the gene product can be analysed. This is achieved by assays based on the physical or functional properties of the product, including radioactive labeling of the product followed by analysis by gel electrophoresis, immunoassay, etc.

The BCMPs identified herein can be isolated and purified by standard methods including chromatography (*e.g.* ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

Alternatively, once a recombinant nucleic acid that encodes the BCMP is identified, the entire amino acid sequence of the BCMP can be deduced from the nucleotide sequence of the gene-coding region contained in the recombinant nucleic acid. As a result, the protein can be synthesized by standard chemical methods known in the art (*e.g.* see Hunkapiller *et al.*, 1984, Nature 310:105-111).

In another alternative embodiment, native BCMPs can be purified from natural sources, by standard methods such as those described above (*e.g.* immunoaffinity purification).

The invention thus provides an isolated BCMP, an isolated BCMP derivative, an isolated BCMP fragment or an isolated BCMP fusion protein; any of the foregoing can be produced by recombinant DNA techniques or by chemical synthetic methods.

<u>Isolation Of DNA Encoding a BCMP</u>

Specific embodiments for the cloning of a gene encoding a BCMP, are presented below by way of example and not of limitation.

The nucleotide sequences of the present invention, including DNA and RNA, and comprising a sequence encoding a BCMP or a fragment thereof, or a BCMP derivative, fragment or fusion protein, may be synthesized using methods known in the art, such as using conventional chemical approaches or polymerase chain reaction (PCR) amplification. The nucleotide sequences of the present invention also permit the identification and cloning of the gene encoding a BCMP homolog or BCMP ortholog including, for example, by screening cDNA libraries, genomic libraries or expression libraries.

For example, to clone a gene encoding a BCMP by PCR techniques, anchored degenerate oligonucleotides (or a set of most likely oligonucleotides) can be designed for all BCMP peptide fragments identified as part of the same protein. PCR reactions under a variety of conditions can be performed with relevant cDNA and genomic DNAs (e.g. from breast tissue or from cells of the immune system) from one or more species. Also vectorette reactions can be performed on any available cDNA and genomic DNA using the oligonucleotides (which preferably are nested) as above. Vectorette PCR is a method that enables the amplification of specific DNA fragments in situations where the sequence of only one primer is known. Thus, it extends the application of PCR to stretches of DNA where the sequence information is only available at one end. (Arnold C, 1991, PCR Methods Appl. 1(1): 39-42; Dyer KD, Biotechniques, 1995, 19(4):550-552). Vectorette PCR may be performed with probes that are, for example, anchored degenerate oligonucleotides (or most likely oligonucleotides) coding for BCMP peptide fragments, using as a template a genomic library or cDNA library pools.

Anchored degenerate oligonucleotides (and most likely oligonucleotides) can be designed for all BCMP peptide fragments. These oligonucleotides may be labeled and hybridized to filters containing cDNA and genomic DNA libraries. Oligonucleotides to different peptides from the same protein will often identify the same members of the library. The cDNA and genomic DNA libraries may be obtained from any suitable or desired mammalian species, for example from humans.

Nucleotide sequences comprising a nucleotide sequence encoding a BCMP or BCMP fragment of the present invention are useful for their ability to hybridize selectively with complementary stretches of genes encoding other proteins. Depending on the application, a variety of hybridization conditions may be employed to obtain nucleotide sequences at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identical, or 100% identical, to the sequence of a nucleotide encoding a BCMP.

For a high degree of selectivity, relatively stringent conditions are used to form the duplexes, such as low salt or high temperature conditions. As used herein, "highly stringent conditions" means hybridization to filter-bound DNA in 0.5 M $NaHPO_4$, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65 °C, and washing in 0.1xSSC/0.1% SDS at 68 °C (Ausubel F.M. *et al.*, eds., 1989, Current Protocols in Molecular Biology, Vol. I, Green Publishing Associates, Inc., and John Wiley & Sons, Inc., New York, at p. 2.10.3). For some applications, less stringent conditions for duplex formation are required. As used herein "moderately stringent conditions" means washing in 0.2xSSC/0.1% SDS at 42 °C (Ausubel *et al.*, 1989, *supra*). Hybridization conditions can also be rendered more stringent by the addition of increasing amounts of formamide, to destabilize the hybrid duplex. Thus, particular hybridization conditions can be readily manipulated, and will generally be chosen depending on the desired results. In general, convenient hybridization temperatures in the presence of 50% formamide

are: 42 °C for a probe which is 95 to 100% identical to the fragment of a gene encoding a BCMP, 37°C for 90 to 95% identity and 32 °C for 70 to 90% identity.

In the preparation of genomic libraries, DNA fragments are generated, some of which will encode parts or the whole of a BCMP. Any suitable method for preparing DNA fragments may be used in the present invention. For example, the DNA may be cleaved at specific sites using various restriction enzymes. Alternatively, one may use DNAse in the presence of manganese to fragment the DNA, or the DNA can be physically sheared, as for example, by sonication. The DNA fragments can then be separated according to size by standard techniques, including but not limited to agarose and polyacrylamide gel electrophoresis, column chromatography and sucrose gradient centrifugation. The DNA fragments can then be inserted into suitable vectors, including but not limited to plasmids, cosmids, bacteriophages lambda or T_4, and yeast artificial chromosome (YAC; See, e.g. Sambrook *et al.*, 1989, Molecular Cloning, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985, DNA Cloning: A Practical Approach, MRL Press, Ltd., Oxford, U.K. Vol. I, II; Ausubel F.M. *et al.*, eds., 1989, Current Protocols in Molecular Biology, Vol. I, Green Publishing Associates, Inc., and John Wiley & sons, Inc., New York). The genomic library may be screened by nucleic acid hybridization to labeled probe (Benton and Davis, 1977, Science 196:180; Grunstein and Hogness, 1975, Proc. Natl. Acad. Sci. USA. 72: 3961).

Based on the present description, the genomic libraries may be screened with labeled degenerate oligonucleotide probes corresponding to the amino acid sequence of any peptide of the BCMP using optimal approaches well known in the art. Any probe used is preferably at least 10 nucleotides, at least 15 nucleotides, at least 20 nucleotides, at least 25 nucleotides, at least 30 nucleotides, at least 40 nucleotides, at least 50 nucleotides, at least 60 nucleotides, at least 70 nucleotides, at least 80 nucleotides, or at least 100 nucleotides.

In Tables 1 and 2 above, some BCMPs disclosed herein were found to correspond to isoforms of previously identified proteins encoded by genes whose sequences are publicly known. (Sequence analysis and protein identification of BCMPs was carried out using the methods described in Examples). To screen such a gene, any probe may be used that is complementary to the gene or its complement; preferably the probe is 10 nucleotides or longer, more preferably 15 nucleotides or longer. The SWISS-PROT and trEMBL databases (held by the Swiss Institute of Bioinformatics (SIB) and the European Bioinformatics Institute (EBI) which are available at http://www.expasy.ch/ and the GenBank database (held by the National Institute of Health (NIH) which is available at http://www.ncbi.nlm.nih.gov/) provide protein sequences for the BCMPs listed in Tables 1 and 2, and each sequence is incorporated herein by reference.

When a library is screened, clones with inserted DNA encoding the BCMP or a fragment thereof will hybridize to one or more members of the corresponding set of degenerate oligonucleotide probes (or their complement). Hybridization of such oligonucleotide probes to genomic libraries is carried out using methods known in the art. For example, hybridization with one of the above-mentioned degenerate sets of oligonucleotide probes, or their complement (or with any member of such a set, or its complement) can be performed under highly stringent or moderately stringent conditions as defined above, or can be carried out in 2X SSC, 1.0% SDS at 50°C and washed using the washing conditions described supra for highly stringent or moderately stringent hybridization.

In yet another aspect of the invention, clones containing nucleotide sequences encoding the entire BCMP, a fragment of a BCMP, BCMP derivative, BCMP fragment or BCMP fusion protein any of the foregoing may also be obtained by screening expression libraries. For example, DNA from the relevant source is isolated and random fragments are prepared and ligated into an expression vector (*e.g.* a bacteriophage, plasmid, phagemid or cosmid) such that the inserted sequence in the vector is capable of being expressed by the host cell into which the vector is then introduced. Various screening assays can then be used to select for the expressed BCMP or BCMP derivative, fragment or fusion proteins. In one embodiment, the various anti-BCMP antibodies of the invention can be used to identify the desired clones using methods known in the art. See, for example, Harlow and Lane, 1988, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, Appendix IV. Colonies or plaques from the library are brought into contact with the antibodies to identify those clones that bind antibody.

In an embodiment, colonies or plaques containing DNA that encodes a BCMP, a fragment of a BCMP, BCMP derivative, BCMP fragment or BCMP fusion protein can be detected using DYNA Beads according to Olsvick *et al.*, 29th ICAAC, Houston, Tex. 1989. Anti-BCMP antibodies are crosslinked to tosylated DYNA Beads M280, and these antibody-containing beads are then contacted with colonies or plaques expressing recombinant polypeptides. Colonies or plaques expressing a BCMP or BCMP-derived polypeptide are identified as any of those that bind the beads.

Alternatively, the anti-BCMP antibodies can be nonspecifically immobilized to a suitable support, such as silica or Celite® resin. This material is then used to adsorb to bacterial colonies expressing the BCMP protein or BCMP derivative, fragment or fusion protein as described herein.

In another aspect, PCR amplification may be used to isolate from genomic DNA a substantially pure DNA (*i.e.*, a DNA substantially free of contaminating nucleic acids) encoding the entire BCMP or a part thereof. Preferably such a DNA is at least 95% pure, more preferably at least 99% pure. Oligonucleotide sequences, degenerate or otherwise, that correspond to peptide sequences of BCMPs disclosed herein can be used as primers.

PCR can be carried out, *e.g.* by use of a Perkin-Elmer Cetus thermal cycler and Taq polymerase (Gene Amp® or AmpliTaq DNA polymerase). One can choose to synthesize several different degenerate primers, for use in the PCR reactions. It is also possible to vary the stringency of hybridization conditions used in priming the PCR reactions, to allow for greater or lesser degrees of nucleotide sequence similarity between the degenerate primers and the corresponding sequences in the DNA. After successful amplification of a segment of the sequence encoding a BCMP, that segment may be molecularly cloned and sequenced, and utilized as a probe to isolate a complete genomic clone. This, in turn, will permit the determination of the gene's complete nucleotide sequence, the analysis of its expression, and the production of its protein product for functional analysis, as described *infra*.

The gene encoding a BCMP can also be identified by mRNA selection by nucleic acid hybridization followed by *in vitro* translation. In this procedure, fragments are used to isolate complementary mRNAs by hybridization. Such DNA fragments may represent available, purified DNA encoding a BCMP of another species (*e.g.* mouse, human). Immunoprecipitation analysis or functional assays (*e.g.* aggregation ability *in vitro*; binding to receptor) of the *in vitro* translation products of the isolated products of the isolated mRNAs identifies the mRNA and, therefore, the complementary DNA fragments that contain the desired sequences. In addition, specific mRNAs may

be selected by adsorption of polysomes isolated from cells to immobilized antibodies that specifically recognize a BCMP. A radiolabeled cDNA encoding a BCMP can be synthesized using the selected mRNA (from the adsorbed polysomes) as a template. The radiolabeled mRNA or cDNA may then be used as a probe to identify the DNA fragments encoding a BCMP from among other genomic DNA fragments.

Alternatives to isolating genomic DNA encoding a BCMP include, but are not limited to, chemically synthesizing the gene sequence itself from a known sequence or making cDNA to the mRNA which encodes the BCMP. For example, RNA for cDNA cloning of the gene encoding a BCMP can be isolated from cells which express the BCMP. Those skilled in the art will understand from the present description that other methods may be used and are within the scope of the invention.

Any suitable eukaryotic cell can serve as the nucleic acid source for the molecular cloning of the gene encoding a BCMP. The nucleic acid sequences encoding the BCMP can be isolated from vertebrate, mammalian, primate, human, porcine, bovine, feline, avian, equine, canine or murine sources. The DNA may be obtained by standard procedures known in the art from cloned DNA (*e.g.* a DNA "library"), by chemical synthesis, by cDNA cloning, or by the cloning of genomic DNA, or fragments thereof, purified from the desired cell. (See, e.g. Sambrook *et al.*, 1989, Molecular Cloning, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985, DNA Cloning: A Practical Approach, MRL Press, Ltd., Oxford, U.K. Vol. I, II). Clones derived from genomic DNA may contain regulatory and intron DNA regions in addition to coding regions; clones derived from cDNA will contain only exon sequences.

The identified and isolated gene or cDNA can then be inserted into any suitable cloning vector. A large number of vector-host systems known in the art may be used. As those skilled in the art will appreciate, the only limitation is that the vector system chosen be compatible with the host cell used. Such vectors include, but are not limited to, bacteriophages such as lambda derivatives, plasmids such as PBR322 or pUC plasmid derivatives or the Bluescript™ vector (Stratagene) or modified viruses such as adenoviruses, adeno-associated viruses or retroviruses. The insertion into a cloning vector can be accomplished, for example, by ligating the DNA fragment into a cloning vector which has complementary cohesive termini. However, if the complementary restriction sites used to fragment the DNA are not present in the cloning vector, the ends of the DNA molecules may be enzymatically modified. Alternatively, any site desired may be produced by ligating nucleotide sequences (linkers) onto the DNA termini; these ligated linkers may comprise specific chemically synthesized oligonucleotides encoding restriction endonuclease recognition sequences. In an alternative method, the cleaved vector and the gene encoding a BCMP may be modified by homopolymeric tailing. Recombinant molecules can be introduced into host cells via transformation, transfection, infection, electroporation, etc., so that many copies of the gene sequence are generated.

In specific embodiments, transformation of host cells with recombinant DNA molecules that incorporate the isolated gene encoding the BCMP, cDNA, or synthesized DNA sequence enables generation of multiple copies of the gene. Thus, the gene may be obtained in large quantities by growing transformants, isolating the recombinant DNA molecules from the transformants and, when necessary, retrieving the inserted gene from the isolated recombinant DNA.

The nucleotide sequences of the present invention include nucleotide sequences encoding amino acid sequences with substantially the same amino acid sequences as native BCMPs, nucleotide

sequences encoding amino acid sequences with functionally equivalent amino acids, nucleotide sequences encoding BCMPs, BCMP derivatives, BCMP fragments or BCMP fusion proteins.

In a specific embodiment, an isolated nucleic acid molecule that encodes a BCMP derivative, fragment or fusion protein, can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of a BCMP such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Standard techniques known to those of skill in the art can be used to introduce mutations, including, for example, site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a side chain with a similar charge. Families of amino acid residues having side chains with similar charges have been defined in the art. These families include amino acids with basic side chains (e.g. lysine, arginine, histidine), acidic side chains (e.g. aspartic acid, glutamic acid), uncharged polar side chains (e.g. glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g. alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g. threonine, valine, isoleucine) and aromatic side chains (e.g. tyrosine, phenylalanine, tryptophan, histidine). Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity. Following mutagenesis, the encoded protein can be expressed and the activity of the protein can be determined.

Expression of DNA Encoding BCMPs

The nucleotide sequence coding for a BCMP, BCMP derivative, BCMP fragment or BCMP fusion protein can be inserted into an appropriate expression vector, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. The necessary signals for transcription and translation can also be supplied by the native gene encoding the BCMP or its flanking regions, or the native gene encoding the BCMP derivative, fragment or fusion protein or its flanking regions. A variety of host-vector systems may be utilized in the present invention to express the protein-coding sequence. These include but are not limited to mammalian cell systems infected with virus (*e.g.* vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (*e.g*, baculovirus); microorganisms such as yeast containing yeast vectors; or bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used. In specific embodiments, a nucleotide sequence encoding a human gene (or a nucleotide sequence encoding a functionally active portion of a human BCMP) is expressed. In yet another embodiment, a fragment of a BCMP comprising a domain of the BCMP is expressed.

Any of the methods previously described for the insertion of DNA fragments into a vector may be used to construct expression vectors containing a chimeric gene consisting of appropriate transcriptional and translational control signals and the protein coding sequences. These methods may include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinants (genetic recombination). Expression of nucleic acid sequence encoding a BCMP or fragment thereof may be

regulated by a second nucleic acid sequence so that the BCMP or fragment is expressed in a host transformed with the recombinant DNA molecule. For example, expression of a BCMP may be controlled by any promoter or enhancer element known in the art. Promoters which may be used to control the expression of the gene encoding a BCMP or a BCMP derivative, fragment or fusion protein include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3'-long terminal repeat of Rous sarcoma virus (Yamamoto, *et al.*, 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner *et al.*, 1981, Proc. Natl. Acad. Sci. USA. 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster *et al.*, 1982, Nature 296:39-42), the tetracycline (Tet) promoter (Gossen *et al.*, 1995, Proc. Nat. Acad. Sci. USA 89:5547-5551); prokaryotic expression vectors such as the □lactamase promoter (Villa-Kamaroff, *et al.*, 1978, Proc. Natl. Acad. Sci. USA. 75:3727-3731), or the *tac* promoter (DeBoer, *et al.*, 1983, Proc. Natl. Acad. Sci. USA. 80: 21-25; see also "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242 :74-94); plant expression vectors comprising the nopaline synthetase promoter region (Herrera-Estrella *et al.*, Nature 303:209-213) or the cauliflower mosaic virus 35S RNA promoter (Gardner, *et al.*, 1981, Nucl. Acids Res. 9: 2871), and the promoter of the photosynthetic enzyme ribulose biphosphate carboxylase (Herrera-Estrella *et al.*, 1984, Nature 310:115-120); promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter, and the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift *et al.*, 1984, Cell 38:639-646; Ornitz *et al.*, 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399-409; MacDonald, 1987, Hepatology 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, Nature 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl *et al.*, 1984, Cell 38:647-658; Adames *et al.*, 1985, Nature 318:533-538; Alexander *et al.*, 1987, Mol. Cell. Biol. 7: 1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder *et al.*, 1986, Cell 45:485-495), albumin gene control region which is active in liver (Pinkert *et al.*, 1987, Genes and Devel. 1: 268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf *et al.*, 1985, Mol. Cell. Biol. 5:1639-1648; Hammer *et al.*, 1987, Science 235:53-58), alpha 1-antitrypsin gene control region which is active in the liver (Kelsey *et al.*, 1987, Genes and Devel. 1:161-171), beta-globin gene control region which is active in myeloid cells (Mogram *et al.*, 1985, Nature 315:338-340; Kollias *et al.*, 1986, Cell 46:89-94; myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead *et al.*, 1987, Cell 48:703-712); myosin light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, Nature 314:283-286); neuronal-specific enolase (NSE) which is active in neuronal cells (Morelli *et al.*, 1999, Gen. Virol. 80: 571-83); brain-derived neurotrophic factor (BDNF) gene control region which is active in neuronal cells (Tabuchi *et al.*, 1998, Biochem. Biophysic. Res. Com. 253:818-823); glial fibrillary acidic protein (GFAP) promoter which is active in astrocytes (Gomes *et al.*, 1999, Braz J Med Biol Res 32(5): 619-631; Morelli *et al.*, 1999, Gen. Virol. 80:571-83) and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason *et al.*, 1986, Science 234:1372-1378).

In a specific embodiment, a vector is used that comprises a promoter operably linked to a BCMP-encoding nucleic acid, one or more origins of replication, and, optionally, one or more selectable markers (*e.g.* an antibiotic resistance gene).

In a specific embodiment, an expression construct is made by subcloning a BCMP or a BCMP derivative, fragment or fusion protein coding sequence into the *Eco*RI restriction site of each of the three pGEX vectors (Glutathione S-Transferase expression vectors; Smith and Johnson, 1988, Gene 7:31-40). This allows for the expression of the BCMP product or BCMP derivative, fragment or fusion protein from the subclone in the correct reading frame.

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the BCMP coding sequence or BCMP derivative, fragment or fusion protein coding sequence may be ligated to an adenovirus transcription/translation control complex, *e.g.* the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.* region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts (see e.g. Logan & Shenk, 1984, Proc. Natl. Acad. Sci. USA 81:355-359). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner *et al.*, 1987, Methods in Enzymol. 153:51-544).

Expression vectors containing inserts of a gene encoding a BCMP or a BCMP derivative, fragment or fusion protein can be identified by three general approaches: (a) nucleic acid hybridization, (b) presence or absence of "marker" gene functions, and (c) expression of inserted sequences. In the first approach, the presence of a gene encoding a BCMP inserted in an expression vector can be detected by nucleic acid hybridization using probes comprising sequences that are homologous to an inserted gene encoding a BCMP. In the second approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (*e.g.* thymidine kinase activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of a gene encoding a BCMP in the vector. For example, if the gene encoding the BCMP is inserted within the marker gene sequence of the vector, recombinants containing the gene encoding the BCMP insert can be identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by assaying the gene product (*i.e.* the inserted BCMP sequence) expressed using the recombinant expression system. Such assays can be based, for example, on the physical or functional properties of the BCMP in *in vitro* assay systems, *e.g.* binding with anti-BCMP antibody.

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus, expression of the genetically engineered BCMP or BCMP derivative, fragment or fusion protein may be controlled. Furthermore, different host cells have characteristic and specific mechanisms for the translational and post-

translational processing and modification (*e.g.* glycosylation, phosphorylation of proteins). Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. For example, expression in a bacterial system will produce an unglycosylated product and expression in yeast will produce a glycosylated product. Eukaryotic host cells that possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T3, and WI38. Furthermore, different vector/host expression systems may effect processing reactions to different extents.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the differentially expressed or pathway gene protein may be engineered. Rather than using expression vectors that contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.* promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched medium, and then are switched to a selective medium. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the differentially expressed or pathway gene protein. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that affect the endogenous activity of the differentially expressed or pathway gene protein.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler, *et al.*, 1977, Cell 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 1962, Proc. Natl. Acad. Sci. USA 48:2026), and adenine phosphoribosyltransferase (Lowy, *et al.*, 1980, Cell 22:817) genes can be employed in tk-, hgprt- or aprt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for dhfr, which confers resistance to methotrexate (Wigler, *et al.*, 1980, Proc. Natl. Acad. Sci. USA 77: 3567; O'Hare, *et al.*, 1981, Proc. Natl. Acad. Sci. USA 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, Proc. Natl. Acad. Sci. USA 78: 2072); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, *et al.*, 1981, J. Mol. Biol. 150: 1) and hygro genes, which confer resistance to hygromycin (Santerre, *et al.*, 1984, Gene 30:147).

In other specific embodiments, the BCMP, fragment, analog, or derivative may be expressed as a fusion, or chimeric protein product (comprising the protein, fragment, analog, or derivative joined via a peptide bond to a heterologous protein sequence). For example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof) resulting in chimeric polypeptides. Such fusion proteins may facilitate purification, increase half-life *in vivo*, and enhance the delivery of an antigen across an epithelial barrier to the immune system. An increase in the half-life *in vivo* and facilitated purification has been shown for chimeric proteins consisting of the first two domains of the human CD-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (see, *e.g.* EP 394827; Traunecker *et al.*, 1988 Nature, 331:84-86). Enhanced delivery of an antigen across the epithelial barrier to the immune system

has been demonstrated for antigens (*e.g*, insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see *e.g*, PCT publications WO 96/22024 and WO 99/04813).

Nucleic acids encoding a BCMP, BCMP derivative or BCMP fragment can be fused to an epitope tag (*e.g.* the hemaglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht *et al.* allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht *et al.*, 1991, Proc. Natl. Acad. Sci. USA 88:8972-897).

Fusion proteins can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding frame, and expressing the chimeric product by methods commonly known in the art. Alternatively, a fusion protein may be made by protein synthetic techniques, *e.g*, by use of a peptide synthesizer.

Both cDNA and genomic sequences can be cloned and expressed.

Domain Structure of BCMPs

Domains of some BCMPs are known in the art and have been described in the scientific literature. Moreover, domains of a BCMP can be identified using techniques known to those of skill in the art. For example, one or more domains of a BCMP can be identified by using one or more of the following programs: ProDom, TMpred, and SAPS. ProDom compares the amino acid sequence of a polypeptide to a database of compiled domains (see, e.g. http://www.toulouse.inra.fr/prodom.html; Corpet F., Gouzy J. & Kahn D., 1999, Nucleic Acids Res., 27:263-267). TMpred predicts membrane-spanning regions of a polypeptide and their orientation. This program uses an algorithm that is based on the statistical analysis of TMbase, a database of naturally occuring transmembrane proteins (see, e.g. http://www.ch.embnet.org/software/TMPRED_form.html; Hofmann & Stoffel. (1993) "TMbase - A database of membrane spanning proteins segments." Biol. Chem. Hoppe-Seyler 347,166). The SAPS program analyzes polypeptides for statistically significant features like charge-clusters, repeats, hydrophobic regions, compositional domains (see, e.g. Brendel *et al.*, 1992, Proc. Natl. Acad. Sci. USA 89: 2002-2006). Thus, based on the present description, the skilled artisan can identify domains of a BCMP having enzymatic or binding activity, and further can identify nucleotide sequences encoding such domains. These nucleotide sequences can then be used for recombinant expression of a BCMP fragment that retains the enzymatic or binding activity of the BCMP.

Based on the present description, the skilled artisan can identify domains of a BCMP having enzymatic or binding activity, and further can identify nucleotide sequences encoding such domains. These nucleotide sequences can then be used for recombinant expression of BCMP fragments that retain the enzymatic or binding activity of the BCMP.

In one embodiment, a BCMP has an amino acid sequence sufficiently similar to domain of a known polypeptide. As used herein, the term "sufficiently similar" refers to a first amino acid or nucleotide sequence which contains a sufficient number of identical or equivalent (e.g. with a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences have or encode a common structural domain or common functional activity or both.

A BCMP domain can be assessed for its function using techniques well known to those of skill in the art. For example, a domain can be assessed for its kinase activity or for its ability to bind to

DNA using techniques known to the skilled artisan. Kinase activity can be assessed, for example, by measuring the ability of a polypeptide to phosphorylate a substrate. DNA binding activity can be assessed, for example, by measuring the ability of a polypeptide to bind to a DNA binding element in a electromobility shift assay.

Production of Antibodies to BCMPs

According to the invention a BCMP, a BCMP derivative, a BCMP fragment or a BCMP fusion protein may be used as an immunogen to generate antibodies which immunospecifically bind such an immunogen. Such immunogens can be isolated by any convenient means, including the methods described above. Antibodies of the invention include, but are not limited to polyclonal, monoclonal, bispecific, humanized or chimeric antibodies, single chain antibodies, Fab fragments and F(ab') fragments, fragments produced by a Fab expression library, anti-diotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above.

In one embodiment, antibodies that recognize gene products of genes encoding BCMPs are publicly available. In another embodiment, methods known to those skilled in the art are used to produce antibodies that recognize a BCMP, BCMP derivative, BCMP fragment or BCMP fusion protein of any of the foregoing.

In one embodiment of the invention, antibodies to a specific domain of a BCMP are produced. In a specific embodiment, hydrophilic fragments of a BCMP are used as immunogens for antibody production.

In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art, *e.g.* ELISA (enzyme-linked immunosorbent assay). For example, to select antibodies that recognize a specific domain of a BCMP, one may assay generated hybridomas for a product which binds to a BCMP fragment containing such domain. For selection of an antibody that specifically binds a first BCMP homolog but which does not specifically bind to (or binds less avidly to) a second BCMP homolog, one can select on the basis of positive binding to the first BCMP homolog and a lack of binding to (or reduced binding to) the second BCMP homolog. Similarly, for selection of an antibody that specifically binds a BCMP but which does not specifically bind to (or binds less avidly to) a different isoform of the same protein (such as a different glycoform having the same core peptide as the BCMP), one can select on the basis of positive binding to the BCMP and a lack of binding to (or reduced binding to) the different isoform (*e.g.* a different glycoform). Thus, the present invention provides an antibody (preferably a monoclonal antibody) that binds with greater affinity (preferably at least 2-fold, more preferably at least 5-fold still more preferably at least 10-fold greater affinity) to a BCMP than to a different isoform or isoforms (*e.g.* glycoforms) of the BCMP.

Polyclonal antibodies which may be used in the methods of the invention are heterogeneous populations of antibody molecules derived from the sera of immunized animals. Unfractionated immune serum can also be used. Various procedures known in the art may be used for the production of polyclonal antibodies to a BCMP, BCMP derivative, BCMP fragment or BCMP fusion protein. In a particular embodiment, rabbit polyclonal antibodies to an epitope of a BCMP or a BCMP derivative, fragment or fusion protein can be obtained. For example, for the production of polyclonal or monoclonal antibodies, various host animals can be immunized by injection with the native or a synthetic (*e.g.* recombinant) version of a BCMP, BCMP derivative, BCMP fragment or BCMP fusion

protein, including but not limited to rabbits, mice, rats, etc. The invention described herein provides isolated BCMPs suitable for such immunization. If the BCMP is purified by gel electrophoresis, the BCMP can be used for immunization with or without prior extraction from the polyacrylamide gel. Various adjuvants may be used to enhance the immunological response, depending on the host species, including, but not limited to, complete or incomplete Freund's adjuvant, a mineral gel such as aluminum hydroxide, surface active substance such as lysolecithin, pluronic polyol, a polyanion, a peptide, an oil emulsion, keyhole limpet hemocyanin, dinitrophenol, and an adjuvant such as BCG (bacille Calmette-Guerin) or corynebacterium parvum. Additional adjuvants are also well known in the art.

For preparation of monoclonal antibodies (mAbs) directed toward a BCMP, BCMP derivative, BCMP fragment or BCMP fusion protein, any technique which provides for the production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole *et al.*, 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAbs of the invention may be cultivated *in vitro* or *in vivo*. In an additional embodiment of the invention, monoclonal antibodies can be produced in germ-free animals utilizing known technology (PCT/US90/02545).

The monoclonal antibodies include but are not limited to human monoclonal antibodies and chimeric monoclonal antibodies (*e.g.* human-mouse chimeras). A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a human immunoglobulin constant region and a variable region derived from a murine mAb (See, *e.g.* Cabilly *et al.*, US Patent No. 4,816,567; and Boss *et al.*, US Patent No. 4,816397) Humanized antibodies are antibody molecules from non-human species having one or more complementarily determining regions (CDRs) from the non-human species and a framework region from a human immunoglobulin molecule (See, *e.g.* Queen, US Patent No. 5,585,089)

Chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in PCT Publication No. WO 87/02671; EP 184187; EP 171496; EP 173494; PCT Publication No. WO 86/01533; US Patent No. 4,816,567; EP 125023; Better *et al.*, 1988, Science 240:1041-1043; Liu *et al.*, 1987, Proc. Natl. Acad. Sci. USA 84: 3439-3443; Liu *et al.*, 1987, J. Immunol. 139:3521-3526; Sun *et al.*, 1987, Proc. Natl. Acad. Sci. USA 84:214-218; Nishimura *et al.*, 1987, Canc. Res. 47:999-1005; Wood *et al.*, 1985, Nature 314:446-449; and Shaw *et al.*, 1988, J. Natl. Cancer Inst. 80:1553-1559; Morrison, 1985, Science 229:1202-1207; Oi *et al.*, 1986, Bio/Techniques 4:214; US Patent 5,225,539; Jones *et al.*, 1986, Nature 321:552-525; Verhoeyan *et al.* (1988) Science 239:1534; and Beidler *et al.*, 1988, J. Immunol. 141:4053-4060.

Completely human antibodies are particularly desirable for therapeutic treatment of human subjects. Such antibodies can be produced using transgenic mice which are incapable of expressing endogenous immunoglobulin heavy and light chain genes, but which can express human heavy and light chain genes. The transgenic mice are immunized in the normal fashion with a selected antigen,

e.g. all or a portion of a BCMP of the invention. Monoclonal antibodies directed against the antigen can be obtained using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar (1995, Int. Rev. Immunol. 13:65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, *see, e.g.* US Patents 5,625,126; 5,633,425; 5,569,825; 5,661,016 and 5,545,806. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies that recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, *e.g.* a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope (Jespers *et al.* (1994) Bio/technology 12:899-903).

The antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (*e.g*, human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, *e.g.* using labeled antigen or antigen bound or captured to a solid surface or bead. The phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman *et al.*, 1995, J. Immunol. Methods 182:41-50; Ames *et al.*, 1995, J. Immunol. Methods 184:177-186; Kettleborough *et al.*, 1994, Eur. J. Immunol. 24:952-58; Persic *et al.*,1997, Gene 187 9-18; Burton *et al.*, 1994, Advances in Immunol, 57:191-280; PCT Application No. PCT/GB91/01134; PCT Publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and US Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g. as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and $F(ab')_2$ fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax *et al.*, 1992, BioTechniques 12(6):864-869; and Sawai *et al.*, 1995, AJRI 34:26-34; and Better *et al.*, 1988, Science 240:1041-1043.

Examples of techniques that can be used to produce single-chain Fvs and antibodies include those described in US Patent Nos. 4,946,778 and 5,258,498; Huston *et al.*, 1991, Methods in

Enzymology 203:46-88; Shu *et al.*, 1993, 90: 7995-999; and Skerra *et al.*, 1998, Science 240:1038-1040.

The invention further provides for the use of bispecific antibodies, which can be made by methods known in the art. Traditional production of full-length bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Milstein *et al.*, 1983, Nature 305:537-539). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, and Traunecker *et al.*, 1991, EMBO J. 10:3655-3659.

According to a different and more preferred approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690. For further details for generating bispecific antibodies see, for example, Suresh *et al.*, Methods in Enzymology, 1986, 121:210.

The invention provides functionally active fragments, derivatives or analogs of the anti-BCMP immunoglobulin molecules. Functionally active means that the fragment, derivative or analog is able to elicit anti-idiotype antibodies (*i.e.*, tertiary antibodies) that recognize the same antigen that is recognized by the antibody from which the fragment, derivative or analog is derived. Specifically, in a preferred embodiment the antigenicity of the idiotype of the immunoglobulin molecule may be enhanced by deletion of framework and CDR sequences that are C-terminal to the CDR sequence that specifically recognizes the antigen. To determine which CDR sequences bind the antigen, synthetic peptides containing the CDR sequences can be used in binding assays with the antigen by any binding assay method known in the art.

The present invention provides antibody fragments such as, but not limited to, $F(ab')_2$ fragments and Fab fragments. Antibody fragments that recognize specific epitopes may be generated by known techniques. $F(ab')_2$ fragments consist of the variable region, the light chain constant region and the CH1 domain of the heavy chain and are generated by pepsin digestion of the antibody molecule. Fab fragments are generated by reducing the disulfide bridges of the $F(ab')_2$ fragments. The invention also provides heavy chain and light chain dimers of the antibodies of the invention, or any minimal fragment thereof such as Fvs or single chain antibodies (SCAs) (*e.g.* as described in US Patent No. 4,946,778; Bird, 1988, Science 242:423-42; Huston *et al.*, 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; and Ward *et al.*, 1989, Nature 334:544-54), or any other molecule with the same specificity as the antibody of the invention. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in *E. coli* may be used (Skerra *et al.*, 1988, Science 242:1038-1041).

In other embodiments, the invention provides fusion proteins of the immunoglobulins of the invention (or functionally active fragments thereof), for example in which the immunoglobulin is fused via a covalent bond (*e.g.* a peptide bond), at either the N-terminus or the C-terminus to an amino acid sequence of another protein (or portion thereof, preferably at least 10, 20 or 50 amino acid portion of the protein) that is not the immunoglobulin. Preferably the immunoglobulin, or fragment thereof, is covalently linked to the other protein at the N-terminus of the constant domain. As stated above, such fusion proteins may facilitate purification, increase half-life *in vivo*, and enhance the delivery of an antigen across an epithelial barrier to the immune system.

The immunoglobulins of the invention include analogs and derivatives that are either modified, *i.e*, by the covalent attachment of any type of molecule as long as such covalent attachment that does not impair immunospecific binding. For example, but not by way of limitation, the derivatives and analogs of the immunoglobulins include those that have been further modified, *e.g.* by glycosylation, acetylation, pegylation, phosphylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, etc. Additionally, the analog or derivative may contain one or more non-classical amino acids.

The foregoing antibodies can be used in methods known in the art relating to the localization and activity of the BCMPs of the invention, *e.g.* for imaging these proteins, measuring levels thereof in appropriate physiological samples, in diagnostic methods, etc.

Expression Of Antibodies

The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or by recombinant expression, and are preferably produced by recombinant expression techniques.

Recombinant expression of antibodies, or fragments, derivatives or analogs thereof, requires construction of a nucleic acid that encodes the antibody. If the nucleotide sequence of the antibody is known, a nucleic acid encoding the antibody may be assembled from chemically synthesized oligonucleotides (*e.g.* as described in Kutmeier *et al.*, 1994, BioTechniques 17:242), which, briefly,

involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding antibody, annealing and ligation of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

Alternatively, the nucleic acid encoding the antibody may be obtained by cloning the antibody. If a clone containing the nucleic acid encoding the particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the antibody may be obtained from a suitable source (*e.g.* an antibody cDNA library, or cDNA library generated from any tissue or cells expressing the antibody) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence.

If an antibody molecule that specifically recognizes a particular antigen is not available (or a source for a cDNA library for cloning a nucleic acid encoding such an antibody), antibodies specific for a particular antigen may be generated by any method known in the art, for example, by immunizing an animal, such as a rabbit, to generate polyclonal antibodies or, more preferably, by generating monoclonal antibodies. Alternatively, a clone encoding at least the Fab portion of the antibody may be obtained by screening Fab expression libraries (*e.g.* as described in Huse *et al.*, 1989, *Science* 246:1275-1281) for clones of Fab fragments that bind the specific antigen or by screening antibody libraries (See, *e.g.* Clackson *et al.*, 1991, Nature 352:624; Hane *et al.*, 1997 Proc. Natl. Acad. Sci. USA 94:4937).

Once a nucleic acid encoding at least the variable domain of the antibody molecule is obtained, it may be introduced into a vector containing the nucleotide sequence encoding the constant region of the antibody molecule (see, *e.g.* PCT Publication WO 86/05807; PCT Publication WO 89/01036; and US Patent No. 5,122,464). Vectors containing the complete light or heavy chain for co-expression with the nucleic acid to allow the expression of a complete antibody molecule are also available. Then, the nucleic acid encoding the antibody can be used to introduce the nucleotide substitution(s) or deletion(s) necessary to substitute (or delete) the one or more variable region cysteine residues participating in an intrachain disulfide bond with an amino acid residue that does not contain a sulfhydyl group. Such modifications can be carried out by any method known in the art for the introduction of specific mutations or deletions in a nucleotide sequence, for example, but not limited to, chemical mutagenesis, in vitro site directed mutagenesis (Hutchinson *et al.*, 1978, J. Biol. Chem. 253:6551), PCR based methods, etc.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison *et al.*, 1984, Proc. Natl. Acad. Sci. 81:851-855; Neuberger *et al.*, 1984, *Nature* 312:604-608; Takeda *et al.*, 1985, Nature 314:452-454) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described *supra*, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human antibody constant region, *e.g.* humanized antibodies.

Once a nucleic acid encoding an antibody molecule of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing the protein of the invention by expressing nucleic acid containing the antibody molecule sequences are described herein. Methods

well known to those skilled in the art can be used to construct expression vectors containing an antibody molecule coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. See, for example, the techniques described in Sambrook *et al.* (1990, Molecular Cloning, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) and Ausubel *et al.* (eds. 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY).

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention.

The host cells used to express a recombinant antibody of the invention may be either bacterial cells such as *Escherichia coli,* or, preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule. In particular, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking *et al.*, 1986, Gene 45:101; Cockett *et al.*, 1990, Bio/Technology 8:2).

A variety of host-expression vector systems may be utilized to express an antibody molecule of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express the antibody molecule of the invention *in situ*. These include but are not limited to microorganisms such as bacteria (*e.g. E. coli, B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (*e.g. Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (*e.g.* baculovirus) containing the antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (*e.g.* cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.* Ti plasmid) containing antibody coding sequences; or mammalian cell systems (*e.g.* COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.* metallothionein promoter) or from mammalian viruses (*e.g.* the adenovirus late promoter; the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions comprising an antibody molecule, vectors that direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther *et al.*, 1983, EMBO J. 2:1791), in which the antibody coding sequence may be ligated individually into the vector in frame with the *lac Z* coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, Nucleic Acids Res. 13:3101-3109; Van Heeke & Schuster, 1989, J. Biol. Chem. 24:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to a matrix such as glutathione-agarose beads followed by elution in the presence of free

glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). In mammalian host cells, a number of viral-based expression systems (*e.g.* an adenovirus expression system) may be utilized.

As discussed above, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.* glycosylation) and processing (*e.g.* cleavage) of protein products may be important for the function of the protein.

For long-term, high-yield production of recombinant antibodies, stable expression is preferred. For example, cells lines that stably express an antibody of interest can be produced by transfecting the cells with an expression vector comprising the nucleotide sequence of the antibody and the nucleotide sequence of a selectable (*e.g.* neomycin or hygromycin), and selecting for expression of the selectable marker. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

The expression levels of the antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3 (Academic Press, New York, 1987). When a marker in the vector system which expresses antibody is amplifiable, an increase in the level of inhibitor present in the culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse *et al.*, 1983, Mol. Cell. Biol. 3:257).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers that enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, 1986, Nature 322:52; Kohler, 1980, Proc. Natl. Acad. Sci. USA 77:2197). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once the antibody molecule of the invention has been recombinantly expressed, it may be purified by any method known in the art for purification of an antibody molecule, for example, by chromatography (*e.g.* ion exchange chromatography, affinity chromatography such as with protein A or specific antigen, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

Alternatively, any fusion protein may be readily purified by utilizing an antibody specific for said fusion protein. For example, a system described by Janknecht *et al.* allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht *et al.*, 1991, Proc. Natl. Acad. Sci. USA 88:8972-897). In this system, the gene of interest is subcloned into a

vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni^{2+} nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

Conjugated Antibodies

In a preferred embodiment, anti-BCMP antibodies or fragments thereof are conjugated to a diagnostic or therapeutic moiety. The antibodies can be used for diagnosis or to determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive nuclides, positron emitting metals (for use in positron emission tomography), and nonradioactive paramagnetic metal ions. See generally US Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; suitable prosthetic groups include streptavidin, avidin and biotin; suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride and phycoerythrin; suitable luminescent materials include luminol; suitable bioluminescent materials include luciferase, luciferin, and aequorin; and suitable radioactive nuclides include I^{125}, I^{131}, In^{111} and Tc^{99}.

Anti-BCMP antibodies or fragments thereof can be conjugated to a therapeutic agent or drug moiety to modify a given biological response. The therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, α-interferon, β-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, a thrombotic agent or an anti-angiogenic agent, *e.g.* angiostatin or endostatin; or, a biological response modifier such as a lymphokine, interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), nerve growth factor (NGF) or other growth factor.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, *e.g.* Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* (eds.), pp 243-56 (Alan R. Liss, Inc 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson *et al.* (eds.), pp 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982).

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in US Patent No. 4,676,980.

An antibody with or without a therapeutic moiety conjugated to it can be used as a therapeutic that is administered alone or in combination with cytotoxic factor(s) and/or cytokine(s).

Diagnosis Of Breast Cancer

In accordance with the present invention, test samples of breast tissue, serum, plasma, lymph nodes, liver, lung and/or bone or urine obtained from a subject suspected of having or known to have breast cancer can be used for diagnosis or monitoring. In one embodiment, a change in the abundance of one or more BCMPs in a test sample relative to a control sample (from a subject or subjects free from breast cancer) or a previously determined reference range indicates the presence of breast cancer. In another embodiment, the relative abundance of one or more BCMPs in a test sample compared to a control sample or a previously determined reference range indicates a subtype of breast cancer (*e.g.* primary or metastatic breast cancer). In yet another embodiment, the relative abundance of one or more BCMPs in a test sample relative to a control sample or a previously determined reference range indicates the degree or severity of breast cancer (e.g. the likelihood for metastasis). In any of the aforesaid methods, detection of one or more BCMPs herein may optionally be combined with detection of one or more additional biomarkers for breast cancer. Any suitable method in the art can be employed to measure the level of BCMPs, including but not limited to 1D-gel electrophoresis described herein, kinase assays, immunoassays to detect and/or visualize the BCMPs (*e.g.* Western blot, immunoprecipitation followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis, immunocytochemistry, etc.). In cases where a BCMP has a known function, an assay for that function may be used to measure BCMP expression. In a further embodiment, a change in the abundance of mRNA encoding one or more BCMPs in a test sample relative to a control sample or a previously determined reference range indicates the presence of breast cancer. Any suitable hybridization assay can be used to detect BCMP expression by detecting and/or visualizing mRNA encoding the BCMP (*e.g.* Northern assays, dot blots, *in situ* hybridization, etc.).

In another embodiment of the invention, labeled antibodies, derivatives and analogs thereof, which specifically bind to a BCMP can be used for diagnostic purposes to detect, diagnose, or monitor breast cancer. Preferably, breast cancer is detected in an animal, more preferably in a mammal and most preferably in a human.

Screening Assays

The invention provides methods for identifying agents (e.g. candidate compounds or test compounds) that bind to a BCMP or have a stimulatory or inhibitory effect on the expression or activity of a BCMP. The invention also provides methods of identifying agents, candidate compounds or test compounds that bind to a BCMP, BCMP derivative, BCMP fragment or a BCMP fusion protein or have a stimulatory or inhibitory effect on the expression or activity of a BCMP, BCMP derivative, BCMP fragment or a BCMP fusion protein. Examples of agents, candidate compounds or test compounds include, but are not limited to, nucleic acids (*e.g.* DNA and RNA), carbohydrates, lipids, proteins, peptides, peptidomimetics, small molecules and other drugs. Agents can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including:

biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, 1997, Anticancer Drug Des. 12:145; US Patent Nos. 5,738,996 and .5,807,683).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.*, 1993, Proc. Natl. Acad. Sci. USA 90:6909; Erb *et al.*, 1994, Proc. Natl. Acad. Sci. USA 91:11422; Zuckermann *et al.*, 1994, J. Med. Chem. 37:2678; Cho *et al.*, 1993, Science 261:1303; Carrell *et al.*, 1994, Angew. Chem. Int. Ed. Engl. 33:2059; Carell *et al.*, 1994, Angew. Chem. Int. Ed. Engl. 33:2061; and Gallop *et al.*, 1994, J. Med. Chem. 37:1233.

Libraries of compounds may be presented, e.g. presented in solution (*e.g.* Houghten, 1992, Bio/Techniques 13:412-421), or on beads (Lam, 1991, Nature 354:82-84), chips (Fodor, 1993, Nature 364:555-556), bacteria (US Patent No. 5,223,409), spores (US Patent Nos. 5,571,698; 5,403,484; and 5,223,409), plasmids (Cull *et al.*, 1992, Proc. Natl. Acad. Sci. USA 89:1865-1869) or phage (Scott and Smith, 1990, Science 249:386-390; Devlin, 1990, Science 249:404-406; Cwirla *et al.*, 1990, Proc. Natl. Acad. Sci. USA 87:6378-6382; and Felici, 1991, J. Mol. Biol. 222:301-310), each of which is incorporated herein in its entirety by reference.

In one embodiment, agents that interact with (*i.e.* bind to) a BCMP, a BCMP derivative, a BCMP fragment (e.g. a functionally active derivative or fragment), or a BCMP fusion protein are identified in a cell-based assay system. A "fusion protein" as used herein, comprises a BCMP, BCMP derivative or BCMP fragment joined to another polypeptide such as , but not limited to glutathione-S-transferase. In accordance with this embodiment, cells expressing a BCMP, a BCMP derivative, a BCMP fragment, or a BCMP fusion protein are contacted with a candidate compound or a control compound and the ability of the candidate compound to interact with the BCMP is determined. If desired, this assay may be used to screen a plurality (e.g. a library) of candidate compounds. The cell, for example, can be of prokaryotic origin (*e.g*, *E. coli*) or eukaryotic origin (*e.g*, yeast or mammalian). Further, the cells can express the BCMP, BCMP derivative, BCMP fragment, or BCMP fusion protein endogenously or be genetically engineered to express said BCMPs, derivatives, fragments or fusion proteins. In certain instances, the BCMP, BCMP derivative, BCMP fragment, or BCMP fusion protein or the candidate compound is labeled, for example with a radioactive label (such as ^{32}P, ^{35}S, and ^{125}I) or a fluorescent label (such as fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde or fluorescamine) to enable detection of an interaction between a BCMP and a candidate compound. The ability of the candidate compound to interact directly or indirectly with a BCMP, a BCMP derivative, a BCMP fragment, or a BCMP fusion protein can be determined by methods known to those of skill in the art. For example, the interaction can be determined by flow cytometry, a scintillation assay, immunoprecipitation or western blot analysis.

In another embodiment, agents that interact with (*i.e.* bind to) a BCMP, a BCMP derivative, a BCMP fragment (e.g. a functionally active derivative or fragment), or a BCMP fusion protein are identified in a cell-free assay system. In accordance with this embodiment, a native or recombinant BCMP, BCMP derivative, BCMP fragment or BCMP fusion protein, is contacted with a candidate compound or a control compound and the ability of the candidate compound to interact with said

BCMPs, derivatives, fragments or fusion proteins is determined. If desired, this assay may be used to screen a plurality (e.g. a library) of candidate compounds. Preferably, the BCMP, derivative, fragment or fusion protein is first immobilized, by, for example, contacting said BCMP, derivative, fragment, or fusion protein with an immobilized antibody which specifically recognizes and binds it, or alternatively by contacting a preparation of the BCMP, derivative, fragment or fusion protein with a surface designed to bind proteins. The BCMP, derivative, fragment, or fusion protein may be partially or completely purified (*e.g.* partially or completely free of other polypeptides) or may be part of a cell lysate. In a particular embodiment the interaction may be a competitive interaction detected in a competitive binding assay. In accordance with this embodiment, cells expressing a BCMP, derivative or fragment or fusion protein are contacted with a candidate compound and a compound known to interact with said BCMP, derivative, fragment or fusion protein. As before, the ability of the candidate compound to interact with a BCMP, derivative, fragment or fusion protein can be determined by methods known to those of skill in the art. These assays, whether cell-based or cell-free, can be used to screen a plurality (e.g. a library) of candidate compounds.

Alternatively, the BCMP, derivative, fragment or fusion protein can be biotinylated using techniques well known to those of skill in the art (*e.g.* biotinylation kit, Pierce Chemicals; Rockford, IL). The ability of the candidate compound to interact with a BCMP, derivative, fragment or fusion protein can be determined by methods known to those of skill in the art.

In another embodiment, a cell-based assay system is used to identify agents that bind to or modulate the activity of a protein, such as an enzyme, or a biologically active portion thereof, which is responsible for the production or degradation of a BCMP or is responsible for the post- translational modification of a BCMP. In a primary screen, a plurality (e.g. a library) of compounds are contacted with cells that naturally or recombinantly express: (i) a BCMP, derivative, fragment or fusion protein, or a biologically active fragment of any of the foregoing; and (ii) a protein that is responsible for processing of the BCMP, derivative, fragment or fusion protein in order to identify compounds that modulate the production, degradation, or post-translational modification of said BCMP, derivative, fragment or fusion protein. If desired, compounds identified in the primary screen can then be assayed in a secondary screen against cells naturally or recombinantly expressing the specific BCMPs of interest. The ability of the candidate compound to modulate the production, degradation or post-translational modification of a BCMP, derivative, fragment or fusion protein can be determined by methods known to those of skill in the art, including without limitation, flow cytometry, a scintillation assay, immunoprecipitation and western blot analysis.

In another embodiment, agents that modulate (*i.e.* upregulate or downregulate) the expression or activity of a BCMP, BCMP derivative, BCMP fragment or BCMP fusion protein are identified by contacting cells (*e.g.* cells of prokaryotic origin or eukaryotic origin) expressing the BCMP, BCMP derivative, BCMP fragment or BCMP fusion protein with a candidate compound or a control compound (*e.g.* phosphate buffered saline; PBS) and determining the expression of that polypeptide, the mRNA encoding the BCMP, the BCMP derivative, the BCMP fragment or the BCMP fusion protein. The level of expression of a selected BCMP, BCMP derivative, BCMP fragment or BCMP fusion protein, mRNA encoding the BCMP, the BCMP derivative, the BCMP fragment or the BCMP fusion protein in the presence of the candidate compound is compared to the level of expression of the BCMP, BCMP derivative, BCMP fragment or BCMP fusion protein, mRNA encoding the BCMP, or

mRNA encoding BCMP derivative, BCMP fragment or BCMP fusion protein in the absence of the candidate compound (*e.g.* in the presence of a control compound). The candidate compound can then be identified as a modulator of the expression of the BCMP, or the BCMP derivative, BCMP fragment or BCMP fusion protein based on this comparison. For example, when expression of the BCMP or mRNA is significantly greater in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of expression of the BCMP or mRNA. Alternatively, when expression of the BCMP or mRNA is significantly less in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of the expression of the BCMP or mRNA. The level of expression of a BCMP or the mRNA that encodes it can be determined by methods known to those of skill in the art. For example, mRNA expression can be assessed by Northern blot analysis or RT-PCR, and protein levels can be assessed by western blot analysis.

In another embodiment, agents that modulate the activity of a BCMP or a BCMP derivative, BCMP fragment or BCMP fusion protein are identified by contacting a preparation containing the BCMP or BCMP derivative, BCMP fragment or BCMP fusion protein or cells (*e.g.* prokaryotic or eukaryotic cells) expressing the BCMP or BCMP derivative, BCMP fragment or BCMP fusion protein with a test compound or a control compound and determining the ability of the test compound to modulate (*e.g.* stimulate or inhibit) the activity of the BCMP or BCMP derivative, BCMP fragment or BCMP fusion protein. The activity of a BCMP or a BCMP derivative, BCMP fragment or BCMP fusion protein can be assessed by detecting induction of a cellular signal transduction pathway of the BCMP or BCMP derivative, BCMP fragment or BCMP fusion protein (*e.g.* intracellular Ca^{2+}, diacylglycerol, IP3, etc.), detecting catalytic or enzymatic activity of the target on a suitable substrate, detecting the induction of a reporter gene (*e.g.* a regulatory element that is responsive to a BCMP or a BCMP derivative, BCMP fragment or BCMP fusion protein and is operably linked to a nucleic acid encoding a detectable marker, *e.g.* luciferase), or detecting a cellular response, for example, cellular differentiation, or cell proliferation. Based on the present description, techniques known to those of skill in the art can be used for measuring these activities (see, *e.g.* US Patent No. 5,401,639). The candidate compound can then be identified as a modulator of the activity of a BCMP or a BCMP derivative, BCMP fragment or BCMP fusion protein by comparing the effects of the candidate compound to the control compound. Suitable control compounds include phosphate buffered saline (PBS) and normal saline (NS).

In another embodiment, agents that modulate (i.e. upregulate or downregulate) the expression, activity or both the expression and activity of a BCMP or BCMP derivative, BCMP fragment or BCMP fusion protein are identified in an animal model. Examples of suitable animals include, but are not limited to, mice, rats, rabbits, monkeys, guinea pigs, dogs and cats. Preferably, the animal used represent a model of breast cancer (*e.g.* xenografts of human breast cancer cell lines such as MDA-MB-345 in oestrogen-deprived Severe Combined Immunodeficient (SCID) mice, Eccles *et al.* 1994 Cell Biophysics 24/25, 279). These can be utilized to test compounds that modulate BCMP levels, since the pathology exhibited in these models is similar to that of breast cancer. In accordance with this embodiment, the test compound or a control compound is administered (*e.g.* orally, rectally or parenterally such as intraperitoneally or intravenously) to a suitable animal and the effect on the expression, activity or both expression and activity of the BCMP or BCMP derivative, BCMP fragment

or BCMP fusion protein is determined. Changes in the expression of a BCMP or BCMP derivative, BCMP fragment or BCMP fusion protein can be assessed by the methods outlined above.

In yet another embodiment, a BCMP or BCMP derivative, BCMP fragment or BCMP fusion protein is used as a "bait protein" in a two-hybrid assay or three hybrid assay to identify other proteins that bind to or interact with a BCMP or BCMP derivative, BCMP fragment or BCMP fusion protein (*see, e.g.* US Patent No. 5,283,317; Zervos *et al.*, 1993, Cell 72:223-232; Madura *et al.*, 1993, J. Biol. Chem. 268:12046-12054; Bartel *et al.*, 1993, Bio/Techniques 14:920-924; Iwabuchi *et al.*, 1993, Oncogene 8:1693-1696; U.S. Patent Nos. 5,283,173, 5,468,614 and 5,667,973; Tirode *et al.*, (1997) J. Biol. Chem. 272(37): 22995-22999; Vidal and Legrain, (1999) Nucleic Acids Research 27(4): 919-929; Legrain and Selig, (2000) FEBS Letters 480: 32-36;WO99/42612 and WO 94/10300). As those skilled in the art will appreciate, such binding proteins are also likely to be involved in the propagation of signals by the BCMPs of the invention as, for example, upstream or downstream elements of a signaling pathway involving the BCMPs of the invention.

This invention further provides novel agents identified by the above-described screening assays and uses thereof for treatments as described herein. In addition, the invention also provides the use of an agent which interacts with, or modulates the activity of, one or more BCMPs of the invention in the manufacture of a medicament for the treatment of breast cancer.

Therapeutic Use of BCMPs

The invention provides for treatment or prevention of various diseases and disorders by administration of a therapeutic compound. Such compounds include but are not limited to: BCMPs, BCMP derivatives, BCMP fragments, BCMP fusion proteins; antibodies to the foregoing; nucleic acids encoding BCMPs, BCMP derivatives, BCMP fragments or BCMP fusion proteins; antisense nucleic acids to a gene encoding a BCMP or BCMP derivative, BCMP fragment or BCMP fusion protein; and modulator (e.g. agonists and antagonists) of a gene encoding a BCMP or BCMP derivative, BCMP fragment or BCMP fusion protein. An important feature of the present invention is the identification of genes encoding BCMPs involved in breast cancer. Breast cancer can be treated (e.g. to ameliorate symptoms or to retard onset or progression) or prevented by administration of a therapeutic compound that promotes function or expression of one or more BCMPs that are decreased in the breast tissue of subjects having breast cancer, or by administration of a therapeutic compound that reduces function or expression of one or more BCMPs that are increased in the breast tissue of subjects having breast cancer.

In one embodiment, one or more antibodies each specifically binding to a BCMP are administered alone or in combination with one or more additional therapeutic compounds or treatments. Examples of such therapeutic compounds or treatments include, but are not limited to, taxol, cyclophosphamide, tamoxifen, and doxorubacin.

Preferably, a biological product such as an antibody is allogeneic to the subject to which it is administered. In a preferred embodiment, a human BCMP or a human BCMP derivative, BCMP fragment or BCMP fusion protein, a nucleotide sequence encoding a human BCMP or a human BCMP derivative, BCMP fragment or BCMP fusion protein, or an antibody to a human BCMPor a human BCMP derivative, BCMP fragment or BCMP fusion protein, is administered to a human subject for therapy (e.g. to ameliorate symptoms or to retard onset or progression) or prophylaxis.

Treatment And Prevention of Breast Cancer

Breast cancer is treated or prevented by administration to a subject suspected of having or known to have breast cancer or to be at risk of developing breast cancer of a compound that modulates (*i.e.*, increases or decreases) the level or activity (*i.e.*, function) of one or more BCMPs that are differentially present in the breast tissue of subjects having breast cancer compared with breast tissue of subjects free from breast cancer. In one embodiment, breast cancer is treated or prevented by administering to a subject suspected of having or known to have breast cancer or to be at risk of developing breast cancer a compound that upregulates (*i.e.*, increases) the level or activity (*i.e.*, function) of one or more BCMPs that are decreased in the breast tissue of subjects having breast cancer. In another embodiment, a compound is administered that downregulates the level or activity (*i.e.*, function) of one or more BCMPs that are increased in the breast tissue of subjects having breast cancer. Examples of such a compound include but are not limited to: BCMPs, BCMP fragments and BCMP derivative, fragment or fusion proteins; nucleic acids encoding a BCMP, a BCMP fragment and a BCMP derivative, fragment or fusion protein (*e.g.* for use in gene therapy); and, for those BCMPs or BCMP derivative, fragment or fusion proteins with enzymatic activity, compounds or molecules known to modulate that enzymatic activity. Other compounds that can be used, *e.g.* BCMP agonists, can be identified using *in vitro* assays.

Breast cancer is also treated or prevented by administration to a subject suspected of having or known to have breast cancer or to be at risk of developing breast cancer of a compound that downregulates the level or activity of one or more BCMPs that are increased in the breast tissue of subjects having breast cancer . In another embodiment, a compound is administered that upregulates the level or activity of one or more BCMPs that are decreased in the breast tissue of subjects having breast cancer. Examples of such a compound include, but are not limited to, BCMP antisense oligonucleotides, ribozymes, antibodies directed against BCMPs, and compounds that inhibit the enzymatic activity of a BCMP. Other useful compounds *e.g.* BCMP antagonists and small molecule BCMP antagonists, can be identified using *in vitro* assays.

In a preferred embodiment, therapy or prophylaxis is tailored to the needs of an individual subject. Thus, in specific embodiments, compounds that promote the level or function of one or more BCMPs are therapeutically or prophylactically administered to a subject suspected of having or known to have breast cancer, in whom the levels or functions of said one or more BCMPs are absent or are decreased relative to a control or normal reference range. In further embodiments, compounds that promote the level or function of one or more BCMPs are therapeutically or prophylactically administered to a subject suspected of having or known to have breast cancer in whom the levels or functions of said one or more BCMPs are increased relative to a control or to a reference range. In further embodiments, compounds that decrease the level or function of one or more BCMPs are therapeutically or prophylactically administered to a subject suspected of having or known to have breast cancer in whom the levels or functions of said one or more BCMPs are increased relative to a control or to a reference range. In further embodiments, compounds that decrease the level or function of one or more BCMPs are therapeutically or prophylactically administered to a subject suspected of having or known to have breast cancer in whom the levels or functions of said one or more BCMPs are decreased relative to a control or to a reference range. The change in BCMP function or level due to

the administration of such compounds can be readily detected, *e.g.* by obtaining a sample (*e.g.* a sample of breast tissue, blood or urine or a tissue sample such as biopsy tissue) and assaying *in vitro* the levels or activities of said BCMPs, or the levels of mRNAs encoding said BCMPs, or any combination of the foregoing. Such assays can be performed before and after the administration of the compound as described herein.

The compounds of the invention include but are not limited to any compound, *e.g.* a small organic molecule, protein, peptide, antibody, nucleic acid, etc. that restores the BCMP profile towards normal. The compounds of the invention may be given in combination with any other compound, including taxol, cyclophosphamide, tamoxifen, and doxorubacin.

Vaccine Therapy

BCMPs may be useful as antigenic material, and may be used in the production of vaccines for treatment or prophylaxis of breast cancer. Such material can be "antigenic" and/or "immunogenic". Generally, "antigenic" is taken to mean that the protein is capable of being used to raise antibodies or indeed is capable of inducing an antibody response in a subject. "Immunogenic" is taken to mean that the protein is capable of eliciting a protective immune response in a subject. Thus, in the latter case, the protein may be capable of not only generating an antibody response but, in addition, non-antibody based immune responses.

The skilled person will appreciate that homologues or derivatives of the BCMPs will also find use as antigenic/immunogenic material. Thus, for instance proteins that include one or more additions, deletions, substitutions or the like are encompassed by the present invention. In addition, it may be possible to replace one amino acid with another of similar "type". For instance, replacing one hydrophobic amino acid with another. One can use a program such as the CLUSTAL program to compare amino acid sequences. This program compares amino acid sequences and finds the optimal alignment by inserting spaces in either sequence as appropriate. It is possible to calculate amino acid identity or similarity (identity plus conservation of amino acid type) for an optimal alignment. A program like BLASTx will align the longest stretch of similar sequences and assign a value to the fit. It is thus possible to obtain a comparison where several regions of similarity are found, each having a different score. Both types of analysis are contemplated in the present invention.

In the case of homologues and derivatives, the degree of identity with a protein as described herein is less important than that the homologue or derivative should retain its antigenicity and/or immunogenicity. However, suitably, homologues or derivatives having at least 60% similarity (as discussed above) with the proteins or polypeptides described herein are provided. Preferably, homologues or derivatives having at least 70% similarity, at least 75% similarity or more preferably at least 80% or at least 85% similarity are provided. Most preferably, homologues or derivatives having at least 90% or even 95% similarity are provided.

In an alternative approach, the homologues or derivatives could be fusion proteins, incorporating moieties that render purification easier, for example by effectively tagging the desired protein or polypeptide. It may be necessary to remove the "tag" or it may be the case that the fusion protein itself retains sufficient antigenicity to be useful.

It is well known that it is possible to screen an antigenic protein or polypeptide to identify epitopic regions, i.e. those regions which are responsible for the protein or polypeptide's antigenicity or

immunogenicity. Methods well known to the skilled person can be used to test fragments and/or homologues and/or derivatives for antigenicity. Thus, the fragments of the present invention should include one or more such epitopic regions or be sufficiently similar to such regions to retain their antigenic/immunogenic properties. Thus, for fragments according to the present invention the degree of identity is perhaps irrelevant, since they may be 100% identical to a particular part of a protein or polypeptide, homologue or derivative as described herein. The key issue, once again, is that the fragment retains the antigenic/immunogenic properties of the protein from which it is derived.

What is important for homologues, derivatives and fragments is that they possess at least a degree of the antigenicity/immunogenicity of the protein or polypeptide from which they are derived. Thus, in an additional aspect of the invention, there is provided antigenic/or immunogenic fragments of a BCMP, or of homologues or derivatives thereof.

The BCMPs, or antigenic fragments thereof, can be provided alone, as a purified or isolated preparation. They may be provided as part of a mixture with one or more other proteins of the invention, or antigenic fragments thereof. In a further aspect, therefore, the invention provides an antigen composition comprising one or more BCMPs of the invention and/or one or more antigenic fragments thereof. Such a composition can be used for the detection and/or diagnosis of breast cancer.

In another aspect, the present invention provides a method of detecting and/or diagnosing breast cancer which comprises:

(i) bringing into contact with a sample to be tested an antigenic BCMP, or an antigenic fragment thereof, or an antigen composition of the invention; and

(ii) detecting the presence of antibodies to breast cancer.

In particular, the protein, antigenic fragment thereof or antigen composition of the present invention can be used to detect IgA, IgM or IgG antibodies. Suitably, the sample to be tested will be a biological sample, for example but not limited to, a sample of blood, breast tissue, lymph node, bone or lung.

In a further aspect, the invention provides the use of an antigenic BCMP, antigenic fragment thereof or an antigenic composition of the present invention in detecting and/or diagnosing breast cancer. Preferably, the detecting and/or diagnosing is carried out *in vitro*.

The antigenic BCMPs, antigenic fragments thereof or antigenic composition of the present invention can be provided as a kit for use in the *in vitro* detection and/or diagnosis of breast cancer. Thus, in a still further aspect, the present invention provides a kit for use in the detection and/or diagnosis of breast cancer, which kit comprises an antigenic BCMP, an antigenic fragment thereof or an antigenic composition of the present invention.

In addition, the antigenic BCMP, antigenic fragment thereof or antigen composition of the invention can be used to induce an immune response against breast cancer. Thus, in a yet further aspect, the invention provides the use of an antigenic BCMP, an antigenic fragment thereof or an antigen composition of the invention in medicine.

In a further aspect, the present invention provides a composition capable of eliciting an immune response in a subject, which composition comprises a BCMP, an antigenic fragment thereof, or an antigen composition of the invention. Suitably, the composition will be a vaccine composition, optionally comprising one or more suitable adjuvants. Such a vaccine composition may be either a prophylactic or therapeutic vaccine composition.

The vaccine compositions of the invention can include one or more adjuvants. Examples well known in the art include inorganic gels, such as aluminium hydroxide, and water-in-oil emulsions, such as incomplete Freund's adjuvant. Other useful adjuvants will be well known to the skilled person.

In yet further aspects, the present invention provides:

(a) the use of a BCMP, an antigenic fragment thereof, or an antigen composition of the invention in the preparation of an immunogenic composition, preferably a vaccine;

(b) the use of such an immunogenic composition in inducing an immune response in a subject; and

(c) a method for the treatment or prophylaxis of breast cancer in a subject, or of vaccinating a subject against breast cancer which comprises the step of administering to the subject an effective amount of a BCMP, at least one antigenic fragment thereof or an antigen composition of the invention, preferably as a vaccine.

In a specific embodiment, the invention provides a preparation of one or more BCMPs or BCMP peptide fragments is used as a vaccine for the treatment of breast cancer. Such preparations may include adjuvants or other vehicles.

In another embodiment, a preparation of oligonucleotides comprising 10 or more consecutive nucleotides complementary to a nucleotide sequence encoding one or more BCMPs or BCMP peptide fragments for use as vaccines for the treatment of breast cancer. Such preparations may include adjuvants or other vehicles.

Gene Therapy

In a specific embodiment, nucleic acids comprising a sequence encoding a BCMP, a BCMP fragment, BCMP derivative, BCMP fragment or BCMP fusion protein, are administered to promote BCMP function by way of gene therapy. Gene therapy refers to administration to a subject of an expressed or expressible nucleic acid. In this embodiment, the nucleic acid produces its encoded polypeptide that mediates a therapeutic effect by promoting BCMP function.

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel *et al.*, 1993, Clinical Pharmacy 12:488-505; Wu and Wu, 1991, Biotherapy 3:87-95; Tolstoshev, 1993, Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan, 1993, Science 260:926-932; and Morgan and Anderson, 1993, Ann. Rev. Biochem. 62:191-217; May, 1993, TIBTECH 11(5):155-215. Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel *et al.*, 1993, Current Protocols in Molecular Biology, John Wiley & Sons, eds., NY; and Kriegler, 1990, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY.

In a preferred aspect, the compound comprises a nucleic acid encoding a BCMP or fragment or chimeric protein thereof, said nucleic acid being part of an expression vector that expresses a BCMP, BCMP derivative, BCMP fragment or BCMP fusion protein in a suitable host. In particular, such a nucleic acid has a promoter operably linked to the BCMP coding region, said promoter being inducible or constitutive (and, optionally, tissue-specific). In another particular embodiment, a nucleic acid molecule is used in which the BCMP coding sequences and any other desired sequences are flanked by

regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the BCMP nucleic acid (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; Zijlstra *et al.*, 1989, Nature 342:435-438).

Delivery of the nucleic acid into a subject may be direct, in which case the subject is directly exposed to the nucleic acid or nucleic acid-carrying vector; this approach is known as *in vivo* gene therapy. Alternatively, delivery of the nucleic acid into the subject may be indirect, in which case cells are first transformed with the nucleic acid *in vitro* and then transplanted into the subject; this approach is known as *ex vivo* gene therapy.

In a specific embodiment, the nucleic acid is directly administered *in vivo*, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, *e.g.* by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.* by infection using a defective or attenuated retroviral or other viral vector (see US Patent No. 4,980,286); by direct injection of naked DNA; by use of microparticle bombardment (*e.g.* a gene gun; Biolistic, Dupont); by coating with lipids, cell-surface receptors or transfecting agents; by encapsulation in liposomes, microparticles or microcapsules; by administering it in linkage to a peptide which is known to enter the nucleus; or by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, *e.g.* Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432), which can be used to target cell types specifically expressing the receptors. In another embodiment, a nucleic acid-ligand complex can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, *e.g.* PCT Publications WO 92/06180, WO 92/22635; WO92/20316, WO93/14188 and WO 93/20221. Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; Zijlstra *et al.*, 1989, Nature 342:435-438).

In a specific embodiment, a viral vector that contains a nucleic acid encoding a BCMP is used. For example, a retroviral vector can be used (see Miller *et al.*, 1993, Meth. Enzymol. 217:581-599). These retroviral vectors have been modified to delete retroviral sequences that are not necessary for packaging of the viral genome and integration into host cell DNA. The nucleic acid encoding the BCMP to be used in gene therapy is cloned into the vector, which facilitates delivery of the gene into a subject. More detail about retroviral vectors can be found in Boesen *et al.*, 1994, Biotherapy 6:291-302, which describes the use of a retroviral vector to deliver the mdr1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes *et al.*, 1994, J. Clin. Invest. 93:644-651; Kiem *et al.*, 1994, Blood 83:1467-1473; Salmons and Gunzberg, 1993, Human Gene Therapy 4:129-141; and Grossman and Wilson, 1993, Curr. Opin. Genetics and Devel. 3:110-114.

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, 1993, Current

Opinion in Genetics and Development 3:499-503 present a review of adenovirus-based gene therapy. Bout *et al.*, 1994, Human Gene Therapy 5:3-10 demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld *et al.*, 1991, Science 252:431-434; Rosenfeld *et al.*, 1992, Cell 68:143-155; Mastrangeli *et al.*, 1993, J. Clin. Invest. 91:225-234; PCT Publication WO94/12649; and Wang, *et al.*, 1995, Gene Therapy 2:775-783.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh *et al.*, 1993, Proc. Soc. Exp. Biol. Med. 204:289-300; US Patent No. 5,436,146).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a subject.

In this embodiment, the nucleic acid is introduced into a cell prior to administration *in vivo* of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, *e.g.* Loeffler and Behr, 1993, Meth. Enzymol. 217:599-618; Cohen *et al.*, 1993, Meth. Enzymol. 217:618-644; Cline, 1985, Pharmac. Ther. 29:69-92) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a subject by various methods known in the art. In a preferred embodiment, epithelial cells are injected, *e.g.* subcutaneously. In another embodiment, recombinant skin cells may be applied as a skin graft onto the subject. Recombinant blood cells (*e.g.* hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, the condition of the subject, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to neuronal cells, glial cells (e.g. oligodendrocytes or astrocytes), epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B-lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g. as obtained from bone marrow, umbilical cord blood, peripheral blood or fetal liver.

In a preferred embodiment, the cell used for gene therapy is autologous to the subject that is treated.

In an embodiment in which recombinant cells are used in gene therapy, a nucleic acid encoding a BCMP is introduced into the cells such that it is expressible by the cells or their progeny, and the recombinant cells are then administered *in vivo* for therapeutic effect. In a specific embodiment, stem

or progenitor cells are used. Any stem or progenitor cells which can be isolated and maintained *in vitro* can be used in accordance with this embodiment of the present invention (see e.g. PCT Publication WO 94/08598; Stemple and Anderson, 1992, Cell 71:973-985; Rheinwald, 1980, Meth. Cell Bio. 21A:229; and Pittelkow and Scott, 1986, Mayo Clinic Proc. 61:771).

In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

Direct injection of a DNA coding for a BCMP may also be performed according to, for example, the techniques described in United States Patent No. 5,589,466. These techniques involve the injection of "naked DNA", *i.e.* isolated DNA molecules in the absence of liposomes, cells, or any other material besides a suitable carrier. The injection of DNA encoding a protein and operably linked to a suitable promoter results in the production of the protein in cells near the site of injection and the elicitation of an immune response in the subject to the protein encoded by the injected DNA. In a preferred embodiment, naked DNA comprising (a) DNA encoding a BCMP and (b) a promoter are injected into a subject to elicit an immune response to the BCMP.

Inhibition of BCMPs to Treat Breast Cancer

In one embodiment of the invention, breast cancer is treated or prevented by administration of a compound that antagonizes (inhibits) the level(s) and/or function(s) of one or more BCMPs which are elevated in the breast tissue of subjects having breast cancer as compared with breast tissue of subjects free from breast cancer.

Compounds useful for this purpose include but are not limited to anti-BCMP antibodies (and fragments and derivatives containing the binding region thereof), BCMP antisense or ribozyme nucleic acids, and nucleic acids encoding dysfunctional BCMPs that are used to "knockout" endogenous BCMP function by homologous recombination (see, *e.g.* Capecchi, 1989, *Science* 244:1288-1292). Other compounds that inhibit BCMP function can be identified by use of known *in vitro* assays, *e.g.* assays for the ability of a test compound to inhibit binding of a BCMP to another protein or a binding partner, or to inhibit a known BCMP function. Preferably such inhibition is assayed *in vitro* or in cell culture, but genetic assays may also be employed. The 1D-gel electrophoresis described herein, can also be used to detect levels of the BCMPs before and after the administration of the compound. Preferably, suitable *in vitro* or *in vivo* assays are utilized to determine the effect of a specific compound and whether its administration is indicated for treatment of the affected tissue, as described in more detail below.

In a specific embodiment, a compound that inhibits a BCMP function is administered therapeutically or prophylactically to a subject in whom an increased breast tissue level or functional activity of the BCMP (*e.g.* greater than the normal level or desired level) is detected as compared with breast tissue of subjects free from breast cancer or a predetermined reference range. Methods standard in the art can be employed to measure the increase in a BCMP level or function, as outlined above. Preferred BCMP inhibitor compositions include small molecules, i.e., molecules of 1000 daltons or less. Such small molecules can be identified by the screening methods described herein.

Antisense Regulation of BCMPs

In a specific embodiment, BCMP expression is inhibited by use of BCMP antisense nucleic acids. The present invention provides the therapeutic or prophylactic use of nucleic acids comprising at least six nucleotides that are antisense to a gene or cDNA encoding a BCMP or a portion thereof. As used herein, a BCMP "antisense" nucleic acid refers to a nucleic acid capable of hybridizing by virtue of some sequence complementarity to a portion of an RNA (preferably mRNA) encoding a BCMP. The antisense nucleic acid may be complementary to a coding and/or noncoding region of an mRNA encoding a BCMP. Such antisense nucleic acids have utility as compounds that inhibit BCMP expression, and can be used in the treatment or prevention of breast cancer.

The antisense nucleic acids of the invention are double-stranded or single-stranded oligonucleotides, RNA or DNA or a modification or derivative thereof, and can be directly administered to a cell or produced intracellularly by transcription of exogenous, introduced sequences.

The invention further provides pharmaceutical compositions comprising an effective amount of the BCMP antisense nucleic acids of the invention in a pharmaceutically acceptable carrier, as described *infra*.

In another embodiment, the invention provides methods for inhibiting the expression of a BCMP nucleic acid sequence in a prokaryotic or eukaryotic cell comprising providing the cell with an effective amount of a composition comprising a BCMP antisense nucleic acid of the invention.

BCMP antisense nucleic acids and their uses are described in detail below.

BCMP Antisense Nucleic Acids

The BCMP antisense nucleic acids are of at least six nucleotides and are preferably oligonucleotides ranging from 6 to about 500 oligonucleotides. In specific aspects, the oligonucleotide is preferably 6-50 nucleotides. The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof and can be single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone. The oligonucleotide may include other appended groups such as peptides; agents that facilitate transport across the cell membrane (see, *e.g.* Letsinger *et al.*, 1989, Proc. Natl. Acad. Sci. USA 86:6553-6556; Lemaitre *et al.*, 1987, Proc. Natl. Acad. Sci. 84:648-652; PCT Publication No. WO 88/09810) or blood-brain barrier (see, *e.g.* PCT Publication No. WO 89/10134); hybridization-triggered cleavage agents (see, *e.g.* Krol *et al.*, 1988, BioTechniques 6:958-976) or intercalating agents (see, *e.g.* Zon, 1988, Pharm. Res. 5:539-549).

In a preferred aspect of the invention, a BCMP antisense oligonucleotide is provided, preferably of single-stranded DNA. The oligonucleotide may be modified at any position on its structure with substituents generally known in the art.

The BCMP antisense oligonucleotide may comprise at least one of the following modified base moieties: 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5-

methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid, wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, 2,6-diaminopurine, and other base analogs.

In another embodiment, the oligonucleotide comprises at least one modified sugar moiety, *e.g.* one of the following sugar moieties: arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the oligonucleotide comprises at least one of the following modified phosphate backbones: a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, a formacetal, or an analog of formacetal.

In yet another embodiment, the oligonucleotide is an alpha-anomeric oligonucleotide. An alpha-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual beta-units, the strands run parallel to each other (Gautier *et al.*, 1987, Nucl. Acids Res. 15:6625-6641).

The oligonucleotide may be conjugated to another molecule, *e.g.* a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent.

Oligonucleotides of the invention may be synthesized by standard methods known in the art, *e.g.* by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein *et al.* (1988, Nucl. Acids Res. 16:3209), and methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin *et al.*, 1988, *Proc. Natl. Acad. Sci. USA* 85:7448-7451).

In a specific embodiment, the BCMP antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector can be introduced *in vivo,* such that it is taken up by a cell; within the cell the vector or a portion thereof is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the BCMP antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the BCMP antisense RNA can be by any promoter known in the art to act in mammalian, preferably human, cells. Such promoters can be inducible or constitutive. Examples of such promoters are outlined above.

The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a gene encoding a BCMP, preferably a human gene encoding a BCMP. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," as referred to herein, means a sequence having sufficient complementarity to be able to hybridize under stringent conditions (*e.g.* highly stringent conditions comprising hybridization in 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65 °C and washing in 0.1xSSC/0.1% SDS at 68 °C, or moderately stringent conditions comprising washing in 0.2xSSC/0.1% SDS at 42°C) with the RNA, forming a stable duplex; in the case of double-stranded BCMP antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be

assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an RNA encoding a BCMP it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Therapeutic Use of BCMP Antisense Nucleic Acids

The BCMP antisense nucleic acids can be used to treat or prevent breast cancer when the target BCMP is overexpressed in the breast tissue of subjects suspected of having or suffering from breast cancer. In a preferred embodiment, a single-stranded DNA antisense BCMP oligonucleotide is used.

Cell types that express or overexpress RNA encoding a BCMP can be identified by various methods known in the art. Such cell types include but are not limited to leukocytes (*e.g.* neutrophils, macrophages, monocytes) and resident cells (*e.g.* astrocytes, glial cells, neuronal cells, and ependymal cells). Such methods include, but are not limited to, hybridization with a BCMP-specific nucleic acid (*e.g.* by Northern hybridization, dot blot hybridization, *in situ* hybridization), observing the ability of RNA from the cell type to be translated *in vitro* into a BCMP, immunoassay, etc. In a preferred aspect, primary tissue from a subject can be assayed for BCMP expression prior to treatment, *e.g.* by immunocytochemistry or *in situ* hybridization.

Pharmaceutical compositions of the invention, comprising an effective amount of a BCMP antisense nucleic acid in a pharmaceutically acceptable carrier, can be administered to a subject having breast cancer.

The amount of BCMP antisense nucleic acid that will be effective in the treatment of breast cancer can be determined by standard clinical techniques.

In a specific embodiment, pharmaceutical compositions comprising one or more BCMP antisense nucleic acids are administered via liposomes, microparticles, or microcapsules. In various embodiments of the invention, such compositions may be used to achieve sustained release of the BCMP antisense nucleic acids. In a specific embodiment, it may be desirable to use liposomes targeted via antibodies to specific identifiable tumor antigens (Leonetti *et al.*, 1990, Proc. Natl. Acad. Sci. USA 87:2448-2451; Renneisen *et al.*, 1990, J. Biol. Chem. 265:16337-16342).

Inhibitory Ribozyme and Triple Helix Approaches

In another embodiment, symptoms of breast cancer may be ameliorated by decreasing the level of a BCMP or BCMP activity by using gene sequences encoding the BCMP in conjunction with well-known gene "knock-out," ribozyme or triple helix methods to decrease gene expression of a BCMP. In this approach ribozyme or triple helix molecules are used to modulate the activity, expression or synthesis of the gene encoding the BCMP, and thus to ameliorate the symptoms of breast cancer. Such molecules may be designed to reduce or inhibit expression of a mutant or non-mutant target gene. Techniques for the production and use of such molecules are well known to those of skill in the art.

Ribozyme molecules designed to catalytically cleave gene mRNA transcripts encoding a BCMP can be used to prevent translation of target gene mRNA and, therefore, expression of the gene product. (See, *e.g.* PCT International Publication WO90/11364; Sarver *et al.*, 1990, Science 247:1222-1225).

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. (For a review, see Rossi, 1994, Current Biology 4, 469-471). The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by an endonucleolytic cleavage event. The composition of ribozyme molecules must include one or more sequences complementary to the target gene mRNA, and must include the well known catalytic sequence responsible for mRNA cleavage. For this sequence, see, *e.g.* US Patent No. 5,093,246.

While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy mRNAs encoding a BCMP, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Myers, 1995, Molecular Biology and Biotechnology: A Comprehensive Desk Reference, VCH Publishers, New York, (see especially Figure 4, page 833) and in Haseloff and Gerlach, 1988, Nature, 334, 585-591.

Preferably the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the mRNA encoding the BCMP, *i.e.*, to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

The ribozymes of the present invention also include RNA endoribonucleases (hereinafter "Cech-type ribozymes") such as the one that occurs naturally in *Tetrahymena thermophila* (known as the IVS, or L-19 IVS RNA) and that has been extensively described by Thomas Cech and collaborators (Zaug, *et al.*, 1984, Science, 224, 574-578; Zaug and Cech, 1986, Science, 231, 470-475; Zaug, *et al.*, 1986, Nature, 324, 429-433; published International patent application No. WO 88/04300 by University Patents Inc.; Been and Cech, 1986, Cell, 47, 207-216). The Cech-type ribozymes have an eight base pair active site which hybridizes to a target RNA sequence whereafter cleavage of the target RNA takes place. The invention encompasses those Cech-type ribozymes which target eight base-pair active site sequences that are present in the gene encoding the BCMP.

As in the antisense approach, the ribozymes can be composed of modified oligonucleotides (*e.g.* for improved stability, targeting, etc.) and should be delivered to cells that express the BCMP *in vivo*. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous mRNA encoding the BCMP and inhibit translation. Because ribozymes, unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficacy.

Endogenous BCMP expression can also be reduced by inactivating or "knocking out" the gene encoding the BCMP, or the promoter of such a gene, using targeted homologous recombination (*e.g.* see Smithies, *et al.*, 1985, Nature 317:230-234; Thomas and Capecchi, 1987, Cell 51:503-512; Thompson *et al.*, 1989, Cell 5:313-321; and Zijlstra *et al.*, 1989, Nature 342:435-438. For example, a mutant gene encoding a non-functional BCMP (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous gene (either the coding regions or regulatory regions of the gene encoding the BCMP) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express the target gene *in vivo*. Insertion of the DNA construct, via

targeted homologous recombination, results in inactivation of the target gene. Such approaches are particularly suited in the agricultural field where modifications to ES (embryonic stem) cells can be used to generate animal offspring with an inactive target gene (*e.g.* see Thomas and Capecchi, 1987 and Thompson, 1989, *supra*). However this approach can be adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors.

Alternatively, the endogenous expression of a gene encoding a BCMP can be reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of the gene (*i.e.*, the gene promoter and/or enhancers) to form triple helical structures that prevent transcription of the gene encoding the BCMP in target cells in the body. (See generally, Helene, 1991, Anticancer Drug Des., 6(6), 569-584; Helene, *et al.*, 1992, Ann. N.Y. Acad. Sci., 660, 27-36; and Maher, 1992, Bioassays 14(12), 807-815).

Nucleic acid molecules to be used in triplex helix formation for the inhibition of transcription should be single stranded and composed of deoxynucleotides. The base composition of these oligonucleotides must be designed to promote triple helix formation via Hoogsteen base pairing rules, which generally require sizeable stretches of either purines or pyrimidines to be present on one strand of a duplex. Nucleotide sequences may be pyrimidine-based, which will result in TAT and CGC triplets across the three associated strands of the resulting triple helix. The pyrimidine-rich molecules provide base complementarity to a purine-rich region of a single strand of the duplex in a parallel orientation to that strand. In addition, nucleic acid molecules may be chosen that are purine-rich, for example, contain a stretch of G residues. These molecules will form a triple helix with a DNA duplex that is rich in GC pairs, in which the majority of the purine residues are located on a single strand of the targeted duplex, resulting in GGC triplets across the three strands in the triplex.

Alternatively, the potential sequences that can be targeted for triple helix formation may be increased by creating a so called "switchback" nucleic acid molecule. Switchback molecules are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex and then the other, eliminating the necessity for a sizeable stretch of either purines or pyrimidines to be present on one strand of a duplex.

In instances wherein the antisense, ribozyme, or triple helix molecules described herein are utilized to inhibit mutant gene expression, it is possible that the technique may so efficiently reduce or inhibit the transcription (triple helix) or translation (antisense, ribozyme) of mRNA produced by normal gene alleles of a BCMP that the situation may arise wherein the concentration of BCMP present may be lower than is necessary for a normal phenotype. In such cases, to ensure that substantially normal levels of activity of a gene encoding a BCMP are maintained, gene therapy may be used to introduce into cells nucleic acid molecules that encode and express the BCMP that exhibit normal gene activity and that do not contain sequences susceptible to whatever antisense, ribozyme, or triple helix treatments are being utilized. Alternatively, in instances whereby the gene encodes an extracellular protein, normal BCMPs can be co-administered in order to maintain the requisite level of BCMP activity.

Anti-sense RNA and DNA, ribozyme, and triple helix molecules of the invention may be prepared by any method known in the art for the synthesis of DNA and RNA molecules, as discussed above. These include techniques for chemically synthesizing oligodeoxyribonucleotides and

oligoribonucleotides well known in the art such as for example solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors that incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

Assays for Therapeutic or Prophylactic Compounds

The present invention also provides assays for use in drug discovery in order to identify or verify the efficacy of compounds for treatment or prevention of breast cancer. Test compounds can be assayed for their ability to restore BCMP levels in a subject having breast cancer towards levels found in subjects free from breast cancer or to produce similar changes in experimental animal models of breast cancer. Compounds able to restore BCMP levels in a subject having breast cancer towards levels found in subjects free from breast cancer or to produce similar changes in experimental animal models of breast cancer can be used as lead compounds for further drug discovery, or used therapeutically. BCMP expression can be assayed by 1D-gel electrophoresis, immunoassays, gel electrophoresis followed by visualization, detection of BCMP activity, or any other method taught herein or known to those skilled in the art. Such assays can be used to screen candidate drugs, in clinical monitoring or in drug development, where abundance of a BCMP can serve as a surrogate marker for clinical disease.

In various specific embodiments, *in vitro* assays can be carried out with cells representative of cell types involved in a subject's disorder, to determine if a compound has a desired effect upon such cell types.

Compounds for use in therapy can be tested in suitable animal model systems prior to testing in humans, including but not limited to rats, mice, chicken, cows, monkeys, rabbits, etc. For *in vivo* testing, prior to administration to humans, any animal model system known in the art may be used. Examples of animal models of breast cancer include, but are not limited to xenografts of human breast cancer cell lines such as MDA-MB-435 in oestrogen-deprived Severe Combined Immunodeficient (SCID) mice (Eccles *et al.*, 1994 Cell Biophysics 24/25, 279). These can be utilized to test compounds that modulate BCMP levels, since the pathology exhibited in these models is similar to that of breast cancer. It is also apparent to the skilled artisan that, based upon the present disclosure, transgenic animals can be produced with "knock-out" mutations of the gene or genes encoding one or more BCMPs. A "knock-out" mutation of a gene is a mutation that causes the mutated gene to not be expressed, or expressed in an aberrant form or at a low level, such that the activity associated with the gene product is nearly or entirely absent. Preferably, the transgenic animal is a mammal, more preferably, the transgenic animal is a mouse.

In one embodiment, test compounds that modulate the expression of a BCMP are identified in non-human animals (*e.g.* mice, rats, monkeys, rabbits, and guinea pigs), preferably non-human animal models for breast cancer, expressing the BCMP. In accordance with this embodiment, a test compound or a control compound is administered to the animals, and the effect of the test compound on expression of one or more BCMPs is determined. A test compound that alters the expression of a BCMP (or a plurality of BCMPs) can be identified by comparing the level of the selected BCMP or

BCMPs (or mRNA(s) encoding the same) in an animal or group of animals treated with a test compound with the level of the BCMP(s) or mRNA(s) in an animal or group of animals treated with a control compound. Techniques known to those of skill in the art can be used to determine the mRNA and protein levels, for example, *in situ* hybridization. The animals may or may not be sacrificed to assay the effects of a test compound.

In another embodiment, test compounds that modulate the activity of a BCMP or a biologically active portion thereof are identified in non-human animals (*e.g.* mice, rats, monkeys, rabbits, and guinea pigs), preferably non-human animal models for breast cancer, expressing the BCMPs. In accordance with this embodiment, a test compound or a control compound is administered to the animals, and the effect of a test compound on the activity of a BCMP is determined. A test compound that alters the activity of a BCMP (or a plurality of BCMPs) can be identified by assaying animals treated with a control compound and animals treated with the test compound. The activity of the BCMP can be assessed by detecting induction of a cellular second messenger of the BCMP (*e.g.* intracellular Ca^{2+}, diacylglycerol, IP3, etc.), detecting catalytic or enzymatic activity of the BCMP or binding partner thereof, detecting the induction of a reporter gene (*e.g.* a regulatory element that is responsive to a BCMP of the invention operably linked to a nucleic acid encoding a detectable marker, such as luciferase or green fluorescent protein), or detecting a cellular response (*e.g.* cellular differentiation or cell proliferation). Techniques known to those of skill in the art can be utilized to detect changes in the activity of a BCMP (see, *e.g.* US Patent No. 5,401,639).

In yet another embodiment, test compounds that modulate the level or expression of a BCMP (or plurality of BCMPs) are identified in human subjects having breast cancer, preferably those having severe breast cancer. In accordance with this embodiment, a test compound or a control compound is administered to the human subject, and the effect of a test compound on BCMP expression is determined by analyzing the expression of the BCMP or the mRNA encoding the same in a biological sample (*e.g.* breast tissue, serum, plasma, or urine). A test compound that alters the expression of a BCMP can be identified by comparing the level of the BCMP or mRNA encoding the same in a subject or group of subjects treated with a control compound to that in a subject or group of subjects treated with a test compound. Alternatively, alterations in the expression of a BCMP can be identified by comparing the level of the BCMP or mRNA encoding the same in a subject or group of subjects before and after the administration of a test compound. Techniques known to those of skill in the art can be used to obtain the biological sample and analyze the mRNA or protein expression. For example, differential gel electrophoresis can be used to assess changes in the level of a BCMP.

In another embodiment, test compounds that modulate the activity of a BCMP (or plurality of BCMPs) are identified in human subjects having breast cancer, (preferably those with severe breast cancer). In this embodiment, a test compound or a control compound is administered to the human subject, and the effect of a test compound on the activity of a BCMP is determined. A test compound that alters the activity of a BCMP can be identified by comparing biological samples from subjects treated with a control compound to samples from subjects treated with the test compound. Alternatively, alterations in the activity of a BCMP can be identified by comparing the activity of a BCMP in a subject or group of subjects before and after the administration of a test compound. The activity of the BCMP can be assessed by detecting in a biological sample (*e.g.* breast tissue, serum, plasma, or urine) induction of a cellular signal transduction pathway of the BCMP (*e.g.* intracellular

Ca^{2+}, diacylglycerol, IP3, etc.), catalytic or enzymatic activity of the BCMP or a binding partner thereof, or a cellular response, for example, cellular differentiation, or cell proliferation. Techniques known to those of skill in the art can be used to detect changes in the induction of a second messenger of a BCMP or changes in a cellular response. For example, RT-PCR can be used to detect changes in the induction of a cellular second messenger.

In a preferred embodiment, a test compound that changes the level or expression of a BCMP towards levels detected in control subjects (*e.g.* humans free from breast cancer) is selected for further testing or therapeutic use. In another preferred embodiment, a test compound that changes the activity of a BCMP towards the activity found in control subjects (*e.g.* humans free from breast cancer) is selected for further testing or therapeutic use.

In another embodiment, test compounds that reduce the severity of one or more symptoms associated with breast cancer are identified in human subjects having breast cancer, preferably subjects with severe breast cancer. In accordance with this embodiment, a test compound or a control compound is administered to the subjects, and the effect of a test compound on one or more symptoms of breast cancer is determined. A test compound that reduces one or more symptoms can be identified by comparing the subjects treated with a control compound to the subjects treated with the test compound. Techniques known to physicians familiar with breast cancer can be used to determine whether a test compound reduces one or more symptoms associated with breast cancer. For example, a test compound that reduces tumour burden in a subject having breast cancer will be beneficial for subjects having breast cancer.

In a preferred embodiment, a test compound that reduces the severity of one or more symptoms associated with breast cancer in a human having breast cancer is selected for further testing or therapeutic use.

Therapeutic and Prophylactic Compositions and their Use

The invention provides methods of treatment (and prophylaxis) comprising administering to a subject an effective amount of a composition comprising a BCMP, BCMP derivative, BCMP fragment or BCMP fusion protein of the invention. In a preferred aspect, said composition is substantially purified (*e.g.* substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human. In a specific embodiment, a non-human mammal is the subject.

Formulations and methods of administration that can be employed when the composition comprises a nucleic acid encoding a BCMP, BCMP derivative, BCMP fragment or BCMP fusion protein are described above; additional appropriate formulations and routes of administration are described below.

Various delivery systems are known and can be used to administer a composition comprising a BCMP, BCMP derivative, BCMP fragment or BCMP fusion protein of the invention, *e.g.* encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the composition comprising a BCMP, BCMP derivative, BCMP fragment or BCMP fusion protein , receptor-mediated endocytosis (see, *e.g.* Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction can be

enteral or parenteral and include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The composition comprising a BCMP, BCMP derivative, BCMP fragment or BCMP fusion protein s may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.* oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, *e.g.* by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved, for example, and not by way of limitation, by local infusion during surgery, topical application, *e.g.* by injection, by means of a catheter, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In one embodiment, administration can be by direct injection into breast tissue or at the site (or former site) of a malignant tumor or neoplastic or pre-neoplastic tissue.

In another embodiment, the composition comprising a BCMP, BCMP derivative, BCMP fragment or BCMP fusion protein can be delivered in a vesicle, in particular a liposome (*see* Langer, 1990, Science 249:1527-1533; Treat *et al.*, in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; *see* generally *ibid.*)

In yet another embodiment, the composition comprising a BCMP, BCMP derivative, BCMP fragment or BCMP fusion protein can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:201; Buchwald *et al.*, 1980, Surgery 88:507; Saudek *et al.*, 1989, New Engl. J. Med. 321:574). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise eds., CRC Press, Boca Raton, Florida, 1974; Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball eds., Wiley, NY, 1984; Ranger and Peppas, J., 1983, Macromol. Sci. Rev. Macromol. Chem. 23:61; see also Levy *et al.*, 1985, Science 228:190; During *et al.*, 1989, Ann. Neurol. 25:351; Howard *et al.*, 1989, J. Neurosurg. 71:105). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, *i.e.*, the breast, thus requiring only a fraction of the systemic dose (see, *e.g.* Goodson, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138 1984).

Other controlled release systems are discussed in the review by Langer (1990, Science 249:1527-1533).

In a specific embodiment where the composition comprises a nucleic acid encoding a BCMP, BCMP derivative, BCMP fragment or BCMP fusion protein of the invention, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.* by use of a retroviral vector (see US Patent No. 4,980,286), or by direct injection, or by use of

microparticle bombardment (*e.g.* a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see *e.g.* Joliot *et al.*, 1991, Proc. Natl. Acad. Sci. USA 88:1864-1868), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a composition comprising a BCMP, BCMP derivative, BCMP fragment or BCMP fusion protein , and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the US Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the composition comprising a BCMP, BCMP derivative, BCMP fragment or BCMP fusion protein , preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the subject. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The composition comprising a BCMP, BCMP derivative, BCMP fragment or BCMP fusion protein s of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the composition comprising a BCMP, BCMP derivative, BCMP fragment or BCMP fusion protein of the invention that will be effective in the treatment of breast cancer can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each subject's circumstances. However, suitable dosage ranges for intravenous administration are generally about 20-500 micrograms of active composition comprising a BCMP, BCMP derivative, BCMP fragment or BCMP fusion protein per kilogram body weight. Suitable dosage ranges for intranasal administration are generally about 0.01pg/kg body weight to 1mg/kg body weight. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

Suppositories generally contain active ingredient in the range of 0.5% to 10% by weight; oral formulations preferably contain 10% to 95% active ingredient.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects (a) approval by the agency of manufacture, use or sale for human administration, (b) directions for use, or both.

Molecular Weight Markers

The invention further provides the use of BCMPs as molecular weight markers

Preferred features of each aspect of the invention are as for each of the other aspects *mutatis mutandis*. The prior art documents mentioned herein are incorporated to the fullest extent permitted by law.

EXAMPLE: Identification of Membrane Proteins Expressed in Breast Cancer Cell Lines

Using the following Reference Protocol, proteins in breast cancer cell line membranes were separated by SDS-PAGE and analysed.

<u>Experimental</u>

1a - Crude fractionation of adherent breast carcinoma cell lines

cell culture

The human breast carcinoma cell lines MDA-MB-468 (ATCC:HB-132), T-47D (ATCC:HB-133), BT-474 (ATCC:HTB-20), and MCF-7 (ATCC:HTB-22) were cultured in DMF12 media

containing 10% foetal calf serum , 2mM glutamine and 1% penicillin/streptomycin. The cells were maintained at 37°C in a humidified atmosphere of 95% air and 5 % CO_2.

1b - Cell fractionation and plasma membrane generation

Adherent cells (2 x 10^8) were washed three times with PBS and scrapped using a plastic cell lifter. Cells were centrifuged at 1000 x g for 5 min at 4°C and the cell pellet was resuspended in homogenization buffer (250 mM Sucrose, 10mM HEPES, 1mM EDTA, 1mM Vanadate and 0.02% azide, protease inhibitors). Cells were fractionated using a ball bearing homogeniser (8.002 mm ball, HGM Lab equipment) until approx. 95% of cells were broken. Membranes were fractionated using the method described by Pasquali *et al*. The fractionated cells were centrifuged at 3000 x g for 10 min at 4°C and the postnuclear supernatant was layered onto a 60% sucrose cushion and centrifuged at 100 000 x g for 45 min. The membranes were collected using a pasteur pipette and layered on a preformed 15 to 60% sucrose gradient and spun at 100 000 x g for 17 hours. Proteins from the fractionated sucrose gradient were run on a 4-20% 1D gel (Novex) and subject to western blotting; those fractions containing alkaline phosphatase and transferrin immunoreactivity but not oxidoreductase II or calnexin immunoreactivity were pooled and represented the plasma membrane fraction.

1c - Preparation of plasma membrane fractions for 1D-gel analysis

Plasma membrane fractions that had transferrin immunoreactivity but no oxidoreductase II or calnexin immunoreactivity were identified. These sucrose fractions were pooled and diluted at least four times with 10mM HEPES, 1mM EDTA 1mM Vanadate, 0.02% Azide. The diluted sucrose fraction was added to a SW40 or SW60 tube and centrifuged at 100 000 x g for 45 min with slow acceleration and deceleration. The supernatant was removed from the membrane pellet and the pellet washed three times with PBS-CM. The membrane pellet was solubulized in 2% SDS in 63mM TrisHCl, pH 7.4. A protein assay was performed followed by the addition of mercaptoethanol (2% final), glycerol (10%) and bromopheneol blue (0.0025% final) was added. A final protein concentration of 1 microgram/microlitre was used for 1D-gel loading.

1d - 1D-gel technology

Protein or membrane pellets were solubilised in 1D-sample buffer (approximately 1mg/ml) and the mixture heated to 95°C for 5 min.

Samples were separated using 1D-gel electrophoresis on pre-cast 8-16% gradient gels purchased from Bio-Rad (Bio-Rad Laboratories, Hemel Hempstead, UK). A sample containing 30-50 micrograms of the protein mixtures obtained from a detergent extract were applied to the stacking gel wells using a micro-pipette. A well containing molecular weight (10, 15, 25, 37, 50, 75, 100, 150 and 250 kDa) was included for calibration by interpolation of the separating gel after imaging. Separation of the proteins was performed by applying a current of 30mA to the gel for approximately 5 hours, or until the bromophenol blue marker dye had reached the bottom of the gel.

After electrophoresis the gel plates were prised open, the gel placed in a tray of fixer (10% acetic acid, 40% ethanol, 50% water) and shaken overnight. The gel was then primed for 30 minutes by shaking in a primer solution (7.5% acetic acid, 0.05% SDS in Milli-Q water) followed by incubation with a fluorescent dye (0.06% OGS dye in 7.5% acetic acid) with shaking for 3 hrs. An

example of a fluorescent dye is disclosed in US Patent No. 6,335,446. Sypro Red (Molecular Probes, Inc., Eugene, Oregon) is a suitable alternative dye for this purpose.

A digital image of the stained gel was obtained by scanning on a Storm Scanner (Molecular Dynamics Inc, USA) in the blue fluorescence mode. The captured image was used to determine the area of the gel to excise for in-gel proteolysis.

1e - Recovery and analysis of selected proteins

Each vertical lane of the gel was excised using either a stainless steel scalpel blade or a PEEK gel cutter that cuts sequentially down the length of the gel lane with no attempt at collecting specific protein bands.

Proteins were processed using in-gel digestion with trypsin (Modified trypsin, Promega, Wisconsin, USA) to generate tryptic digest peptides. Recovered samples were divided into two. Prior to MALDI analysis samples were desalted and concentrated using C18 Zip Tips™ (Millipore, Bedford, MA). Samples for tandem mass spectrometry were purified using a nano LC system (LC Packings, Amsterdam, The Netherlands) incorporating C18 SPE material. Recovered peptide pools were analysed by MALDI-TOF-mass spectrometry (Voyager STR, Applied Biosystems, Framingham, MA) using a 337 nm wavelength laser for desorption and the reflectron mode of analysis. Pools were also analyzed by nano-LC tandem mass spectrometry (LC/MS/MS) using a Micromass Quadrupole Time-of-Flight (Q-TOF) mass spectrometer (Micromass, Altrincham, UK). For partial amino acid sequencing and identification of BCMPs uninterpreted tandem mass spectra of tryptic peptides were searched against a database of public domain proteins constructed of protein entries in the non-redundant database held by the National Centre for Biotechnology Information (NCBI) which is accessible at http://www.ncbi.nlm.nih.gov/ using the SEQUEST search program (Eng *et al.*, 1994, J. Am. Soc. Mass Spectrom. 5:976-989), version v.C.1. Criteria for database identification included: the cleavage specificity of trypsin; the detection of a suite of a, b and y ions in peptides returned from the database, and a mass increment for all Cys residues to account for carbamidomethylation. Following identification of proteins through spectral-spectral correlation using the SEQUEST program, masses detected in MALDI-TOF mass spectra were assigned to tryptic digest peptides within the proteins identified. In cases where no amino acid sequences could be identified through searching with uninterpreted MS/MS spectra of tryptic digest peptides using the SEQUEST program, tandem mass spectra of the peptides were interpreted manually, using methods known in the art. (In the case of interpretation of low-energy fragmentation mass spectra of peptide ions see Gaskell *et al.*, 1992, Rapid Commun. Mass Spectrom. 6:658-662). The method described in PCT Application No. PCT/GB01/04034 was also used to interpret mass spectra.

RESULTS

These initial experiments identified 131 BCMPs that matched conceptual translations of cDNAs for which no protein or biological function has been described (Table 1) and 414 BCMPs which matched known proteins not previously been described in breast cell membranes (Table 2).

All references herein are incorporated in their entirety.

CLAIMS:

1. A method of treating or preventing breast cancer comprising administering to a subject one or more BCMPs as defined in Table 1 or 2 herein, or one or more fragments or derivatives thereof.

2. The use of one or more BCMPs as defined in Table 1 or 2 herein, one or more fragments or derivatives thereof, in the manufacture of a medicament for the treatment or prophylaxis of breast cancer.

3. A vaccine comprising one or more BCMPs as defined in Table 1 or 2 herein or derivatives thereof, and/or one or more antigenic or immunogenic fragments thereof.

4. A composition capable of eliciting an immune response in breast cancer in a subject, which composition comprises one or more BCMPs as defined in Table 1 or 2 herein and/or one or more antigenic or immunogenic fragments thereof, and one or more suitable adjuvants.

5. The use of a composition as claimed in claim 4 in inducing an immune response in a subject.

6. The use of one or more BCMPs as defined in Table 1 or 2 herein and/or one or more antigenic or immunogenic fragments thereof, in the preparation of an immunogenic composition, preferably a vaccine.

7. A method for the treatment or prophylaxis of breast cancer in a subject, or of vaccinating a subject against breast cancer, which comprises the step of administering to the subject an effective amount of one or more BCMPs as defined in Table 1 or 2 herein and/or one or more antigenic or immunogenic fragments thereof, as a vaccine.

8. A method of detecting, diagnosing and/or screening for breast cancer which comprises:
(a) bringing into contact with a sample to be tested one or more BCMPs as defined in Table 1 or 2 herein, or one or more antigenic or immunogenic fragments thereof; and
(b) detecting the presence of antibodies to breast cancer.

9. The use of one or more BCMPs as defined in Table 1 or 2 herein and/or one or more antigenic or immunogenic fragments thereof, in screening for, detecting and/or diagnosing breast cancer.

10. A kit for use in the screening for, detection and/or diagnosis of breast cancer, which kit comprises one or more BCMPs as defined in Table 1 or 2 herein and/or one or more antigenic or immunogenic fragments thereof.

11. An antibody capable of immunospecific binding to a BCMP as defined in Table 1 or 2 herein.

12. A kit comprising an antibody as defined in claim 11.

13. A kit comprising a plurality of distinct antibodies as defined in claim 11.

14. A pharmaceutical composition comprising a therapeutically effective amount of an antibody as defined in claim 11, or a fragment or derivative thereof which comprises the binding domain of the antibody, and optionally a pharmaceutically acceptable carrier.

15. A method of treating or preventing breast cancer comprising administering to a subject an antibody as defined in claim 11, or a fragment or derivative thereof which comprises the binding domain of the antibody.

16. The use of an antibody as defined in claim 11, a fragment or derivative thereof which comprises the binding domain of the antibody, in the manufacture of a medicament for the treatment of breast cancer.

17. A method of treating or preventing breast cancer comprising administering to a subject in need of such treatment or prevention a therapeutically effective amount of nucleic acid encoding one or more BCMPs as defined in Table 1 or 2 herein or one or more fragments or derivatives thereof.

18. A method of treating or preventing breast cancer comprising administering to a subject in need of such treatment or prevention a therapeutically effective amount of nucleic acid that inhibits the function or expression of one or more BCMPs as defined in Table 1 or 2 herein.

19. The method of claim 18, wherein the nucleic acid is a BCMP anti-sense nucleic acid or ribozyme.

20. The use of a nucleic acid encoding one or more BCMPs as defined in Table 1 or 2 herein or one or more fragments or derivatives thereof, in the manufacture of a medicament for treating or preventing breast cancer.

21. The use of nucleic acid that inhibits the function or expression of one or more BCMPs as defined in Table 1 or 2 herein, in the manufacture of a medicament for treating or preventing breast cancer.

22. The use of claim 21, wherein the nucleic acid is a BCMP anti-sense nucleic acid or ribozyme.

23. A method for screening for and/or diagnosis of breast cancer in a human subject, which comprises the step of identifying the presence or absence of one or more BCMPs as defined in Table 1 or 2 herein in a biological sample obtained from said human subject.

24. A method for monitoring and/or assessing breast cancer treatment in a human subject, which comprises the step of identifying the presence or absence of one or more BCMPs as defined in Table 1 or 2 herein in a biological sample obtained from said human subject.

25. A method for identifying the presence or absence of metastatic breast cancer cells in a biological sample obtained from a human subject, which comprises the step of identifying the presence or absence of one or more BCMPs as defined in Table 1 or 2 herein.

26. A method for monitoring and/or assessing breast cancer treatment in a human subject, which comprises the step of determining whether one or more BCMPs as defined in Table 1 or 2 herein is increased/decreased in a biological sample obtained from a patient.

27. A method as claimed in any one of claims 23 to 26, wherein the method comprises an immunoassay step utilising one or more antibodies against the or each BCMP.

28. A method as claimed in any one of claims 23 to 26, wherein the method comprises the use of nucleic acid probes and/or PCR reactions to amplify nucleic acid coding for the or each BCMP.

29. A method as claimed in any one of claims 23 to 26, wherein a whole body scan of the subject is carried out to determine localisation of breast cancer cells, particularly metastatic breast cancer cells.

30. A method as claimed in claim 29, wherein labelled antibodies are employed.

31. A diagnostic kit comprising one or more reagents for use in the detection and/or determination of one or more BCMPs as defined in Table 1 or 2 herein.

32. A kit as claimed in claim 31, which comprises one or more containers with one or more antibodies against one or more BCMPs.

33. A kit as claimed in claim 32, which further comprises a labelled binding partner to the or each antibody and/or a solid phase upon which the or each antibody is/are immobilised.

34. A kit as claimed in claim 32 which comprises a nucleic acid probe capable of hybridizing to DNA or RNA encoding the or each BCMP.

35. A method for screening, diagnosis or prognosis of breast cancer in a subject or for monitoring the effect of an anti-breast cancer drug or therapy administered to a subject, comprising: in a sample from the subject, quantitatively detecting one or more BCMPs as defined in Table 1 or 2 herein.

36. The method according to claim 35, wherein the sample is a sample of breast tissue.

37. The method according to claim 35 or 36, wherein the step of quantitatively detecting comprises testing the sample, said step of testing comprising:

(a) contacting the sample with a capture reagent to capture the BCMP; and
(b) detecting the captured BCMP using a directly or indirectly labelled detection reagent.

38. The method according to claim 37, wherein the capture reagent is an antibody.

39. The method according to claim 38, wherein the step of quantitatively detecting comprises testing the sample with a plurality of antibodies for quantitative detection of a plurality of preselected BCMPs.

40. The method according to any one of claims 37 or 39, wherein the BCMP is an isoform and the capture reagent recognises the component part of that isoform which distinguishes the isoform from other members of the gene family, lectin for carbohydrate, or phosphotyrosine or phosphoserine/threonine Ab, or methylation or acetylation Ab.

41. The method according to any one of claims 38 to 40, wherein each antibody is a monoclonal antibody.

42. A method of screening for compounds that interact with a BCMP as defined in Table 1 or 2 herein or biologically active portion thereof, the method comprising:

contacting said BCMP or biologically active portion thereof with a candidate compound; and

determining the ability of the candidate compound to interact with the BCMP or biologically active portion thereof.

43. A method of screening for or identifying compounds that modulate the activity of a BCMP as defined in Table 1or 2 herein or biologically active portion thereof, the method comprising:

in a first aliquot, contacting a candidate compound with the BCMP or biologically active portion thereof; and

comparing the activity of the BCMP or biologically active portion thereof in the first aliquot after addition of the candidate compound with the activity of the BCMP or biologically active portion thereof in a control aliquot, or with a previously determined reference range.

44. The method of claim 42 or 43, wherein the BCMP or biologically active portion thereof is expressed by a cell.

45. The method of any of claims 42 to 44, wherein the BCMP or biologically active portion thereof is recombinant.

46. The method of claim 45, wherein the BCMP or biologically active portion thereof is immobilised on a solid phase.

47. A method of screening for compounds that modulate the expression or activity of a BCMP as defined in Table 1 or 2 herein, comprising:

contacting an enzyme which is responsible for the production or degradation of said BCMP with a candidate compound;

detecting modulation of the activity of said enzyme.

48. A method of screening for compounds that modulate the expression or activity of a BCMP as defined in Table 1 or 2 herein, comprising:

contacting a first group of cells expressing said BCMP with a candidate compound;

contacting a second group of cells expressing said BCMP with a control compound; and

comparing the level of said BCMP or mRNA encoding said BCMP in the first and second groups of cells, or comparing the level of induction of a cellular second messenger in the first and second groups of cells.

49. A method of screening for or identifying compounds that modulate the expression or activity of a BCMP as defined in Table 1 or 2 herein, the method comprising:

administering a candidate compound to a first group of mammals;

administering a control compound to a second group of mammals; and

comparing the level of expression of the BCMP or of mRNA encoding the BCMP in the first and second groups, or comparing the level of induction of a cellular second messenger in the first and second groups.

50. The method of claim 49, wherein the mammals are animal models for breast cancer.

51. A method for screening, diagnosis or prognosis of breast cancer in a subject or for monitoring the effect of an anti-breast cancer drug or therapy administered to a subject, comprising:

(a) contacting one or more oligonucleotide probes comprising 10 or more consecutive nucleotides complementary to a nucleotide sequence encoding one or more BCMPs as defined in Table 1 or 2 herein with an RNA obtained from a biological sample from the subject or with cDNA copied from the RNA, wherein said contacting occurs under conditions that permit hybridization of the probe to the nucleotide sequence if present;

(b) detecting hybridization, if any, between the probe and the nucleotide sequence; and

(c) comparing the hybridization, if any, detected in step (b) with the hybridization detected in a control sample, or with a previously determined reference range.

52. The method of claim 53, wherein step (a) includes the step of hybridizing the nucleotide sequence to a nucleotide, preferably DNA, array, wherein one or more members of the array are the probes complementary to a plurality of nucleotide sequences encoding distinct BCMPs.

53. The use of an agent which interacts with, or modulates the activity of one or more BCMPs defined in Table 1 or 2 in the manufacture of a medicament for the treatment of breast cancer.

ABSTRACT

PROTEINS

The present invention provides methods and compositions for screening, diagnosis and prognosis of breast cancer, for monitoring the effectiveness of breast cancer treatment, and for drug development.